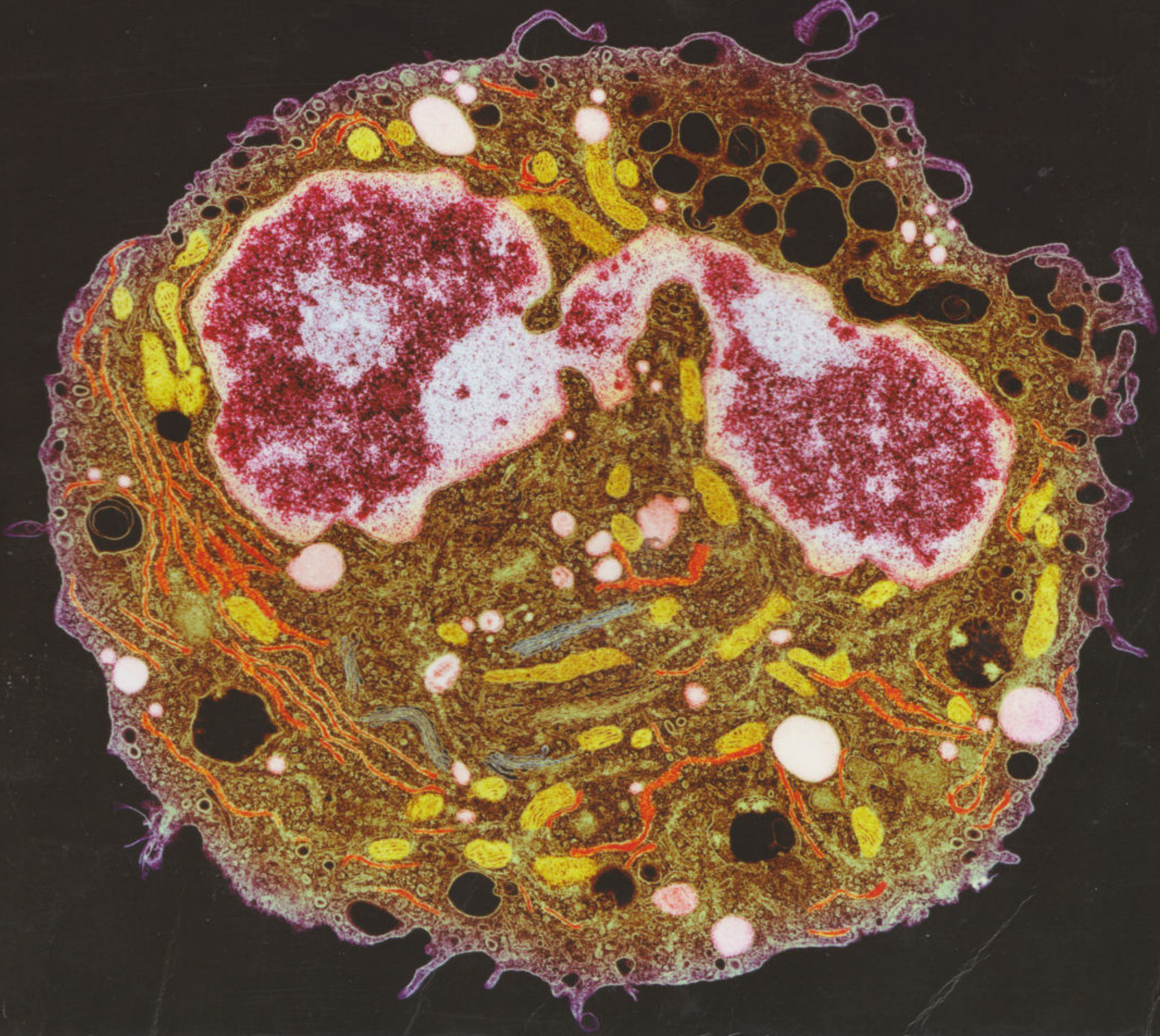
S294 Cell biology
Science: Level 2

The Open University

Book 2
Working Cells



The Open University

S294 Cell biology

Book 2
Working Cells

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Chapter 1 Proteins

1.1 Introducing proteins

Proteins are common to *all* living organisms and their activities and functions are essential for life. As you saw in Book 1, proteins are produced as a result of gene expression, i.e. by the interpretation of the DNA sequence in a gene, through the processes of transcription and translation. The human genome contains 20 000–25 000 protein-coding genes and it is estimated that an individual human cell contains about 10 billion protein molecules of approximately 10 000 different varieties. These figures highlight the point made in Chapter 6 of Book 1, that only a subset of genes are expressed in a cell at any given time and the control of gene expression is a primary means by which cell morphology, processes, activities and functions are regulated.

■ From what you learnt in Section 6.5 in Book 1, why is it likely that the human genome encodes many more proteins than it contains protein-encoding genes?

□ Alternative splicing of RNA transcripts means that one gene may encode more than one protein. In the human genome, on average each open reading frame (ORF) produces two or three different proteins. Post-translational modification of polypeptides further accounts for the variety in proteins.

■ What are the units (monomers) from which proteins are built?

□ Proteins are polymers of amino acids. The amino acid monomers are linked together in a long unbranched chain (Book 1, Sections 1.1 and 6.6).

Some proteins contain more than one chain of amino acids, termed a polypeptide. Most polypeptides are between ~40 and 5000 amino acids long and have relative molecular masses (M_r) ranging from ~4000 to over 580 000 (see Box 1.1), though some exceptional polypeptides are very large: for example, titin, a cytoskeletal protein found in skeletal and cardiac muscle cells of vertebrates, is a polypeptide of 30 000 amino acids with an M_r of 3 500 000!

Box 1.1 Relative molecular mass

The **relative molecular mass (M_r)** of a molecule, such as a protein, is the ratio of the mass of that molecule to 1/12 the mass of an atom of carbon-12. This value can be calculated as the sum of the relative atomic masses of all the atoms that the molecule contains (where the relative atomic mass of an atom is the ratio of its mass to 1/12 the mass of an atom of carbon-12). Being a relative measure, M_r has no units (it is 'dimensionless'). Note, however, that relative molecular mass is numerically equivalent to *molecular mass* which is given in units of daltons (Da) or kilodaltons (kDa, equivalent to 1000 Da) and it has become more common to indicate protein size in this way.

Proteins are involved in every cellular process. In this module, you will encounter many different proteins in all aspects of cell biology. To help you appreciate the huge variety of proteins and their functions, the major broad categories are outlined below.

Enzymes

Enzymes are proteins with catalytic properties, capable of increasing the rate at which chemical reactions occur; they account for the vast majority of proteins. Enzymes are described in detail in Section 1.8.

Structural proteins

There are many different types of structural protein, including those that make up the cytoskeleton (described in Section 3.4.2 of Book 1), which gives the cell its shape and mechanical strength.

■ What are the proteins that make up microfilaments and microtubules?

□ Microfilaments are composed of actin and microtubules are composed of tubulin.

As you have already learnt, these protein molecules self-associate to form the long filaments of the cytoskeleton. In multicellular organisms, protein components of the extracellular matrix (i.e. outside the cell) offer mechanical support. Collagen, of which there are several types, is the most abundant extracellular protein in animal tissues and provides strength. Elastin confers elastic properties to the extracellular matrix of tissues such as blood vessels.

Adhesion proteins

Proteins at the cell surface mediate contact between cells or between a cell and its extracellular environment. In multicellular organisms, these contacts are essential for the maintenance of tissues and are particularly important during development in many organisms.

Signalling proteins

Cells may produce a variety of different signalling proteins which act as signals to other cells or even to the cell from which they originated. Many unicellular organisms communicate with each other through signalling proteins released from their cell surface. Such intercellular signalling proteins are recognised by the 'receiving' cell by means of specific receptor proteins on the cell membrane (see below). Signalling proteins and their receptors are described in detail in Chapter 4.

Receptor proteins

Receptor proteins recognise intercellular signals and other molecules on the surface of neighbouring cells or within the extracellular matrix. Cell surface receptor proteins typically bind an extracellular signalling molecule and communicate with the interior of the cell where a response is initiated. Intracellular receptor proteins are located inside the cell and bind to signalling molecules that can cross the cell membrane.

Transport proteins

The efficient transport of substances within the cell, as well as to and from the cell in some cases, is facilitated by binding to a specific transport protein. Some proteins located within membranes (particularly the cell membrane) permit or facilitate the movement of substances across the membrane. Membrane transport mechanisms are described in detail in the next chapter.

Motor proteins

Motor proteins generate movement in a cell. They move vesicles along microtubules, to deliver the enclosed contents to where they are required or discharged. In muscle cells, the interactions and activities of motor proteins are essential for contraction. Motor proteins are described in Chapter 5.

Immunoglobulins

Immunoglobulins (antibodies; see Book 1, Section 2.2.3) recognise specific 'foreign' targets called antigens (usually also proteins) on the surface of infected cells or bacteria. This is an important part of the immune response.

Storage proteins

Some proteins bind to and serve as a storage facility for nutrients. For example, ferritin, a protein found in animals, plants, bacteria and algae, is an iron storage protein.

Gene regulatory proteins

As you learnt in Sections 6.3 and 6.4 of Book 1, a number of different proteins can bind to DNA to control expression of genes.

■ From your studies so far, give some examples of gene regulatory proteins.

□ Examples that you may have thought of are the *lac* repressor and the catabolite activator protein (CAP) in prokaryotic cells; and TATA-binding protein (TBP, part of transcription factor TFIID), Sp1, heat shock factor and MyoD1 in eukaryotic cells (Book 1, Table 6.2).

There are many examples of proteins that fall into more than one of the above categories. For example, motor proteins and many receptor proteins also have enzymatic activity. Less obviously, proteins involved in intercellular adhesion, through interactions with cytoskeletal proteins, play a crucial structural role in the cell.

■ Many cellular processes involve large assemblies of proteins in complexes, often with other macromolecules. From your studies so far, can you think of any such complexes?

□ Ribosomes, which are responsible for the translation of mRNA transcript and protein synthesis, are large complexes of proteins and RNA molecules (Section 3.4.4 of Book 1). In prokaryotic cells, ribosomes contain more than 50 proteins; in eukaryotic cells, they contain more than 80 proteins.

The diversity of structure and function among proteins is truly impressive, as you will see throughout this module. In this chapter, the nature and structure of proteins are explored in some detail and emphasis is placed on how a protein's structure determines its function or activity. All proteins, in carrying out their specific functions, need to bind to small molecules, ions or macromolecules (including other proteins), which in this context are referred to as **ligands**. Importantly, protein–ligand interactions are always specific; that is, the structure of the protein is such that it will bind only one particular ion or molecule or one of a number of chemically and structurally similar molecules.

The importance of structure in protein function is evident from the study of a number of diseases in which accumulation of inappropriately structured proteins is a major pathological feature (Section 1.5.5). As you will see, the key to the function of the majority of proteins is their interaction with ligands; and regulation of these interactions, as well as other activities, is an essential aspect of how the cell regulates processes in response to environmental changes and signals. Within the cell, the regulation of the activity of a protein and its capacity to interact with other cellular components is achieved through a variety of structural alterations and manipulations, some subtle, some not so subtle, and these mechanisms are described later in this chapter. Enzymes, whose function it is to catalyse reactions, collectively represent the majority of proteins in the cell, and a substantial part of this chapter (Section 1.8) focuses on these proteins.

Summary of Section 1.1

- Proteins are common to all living organisms and are involved in every cellular process. The diversity of function in proteins is reflected in their structural diversity.
- A protein interacts with a limited number of specific ligands and its structure determines what ligands it can interact with. Protein–ligand interactions are essential to protein function.

1.2 The structural hierarchy of proteins

The key to the function of a protein lies in its structure, which in turn depends on the linear sequence of the particular amino acids of which the protein is composed and the interactions between these amino acids.

Protein structure is described in terms of four levels of organisation, termed primary, secondary, tertiary and quaternary, and these are represented in Figure 1.1.

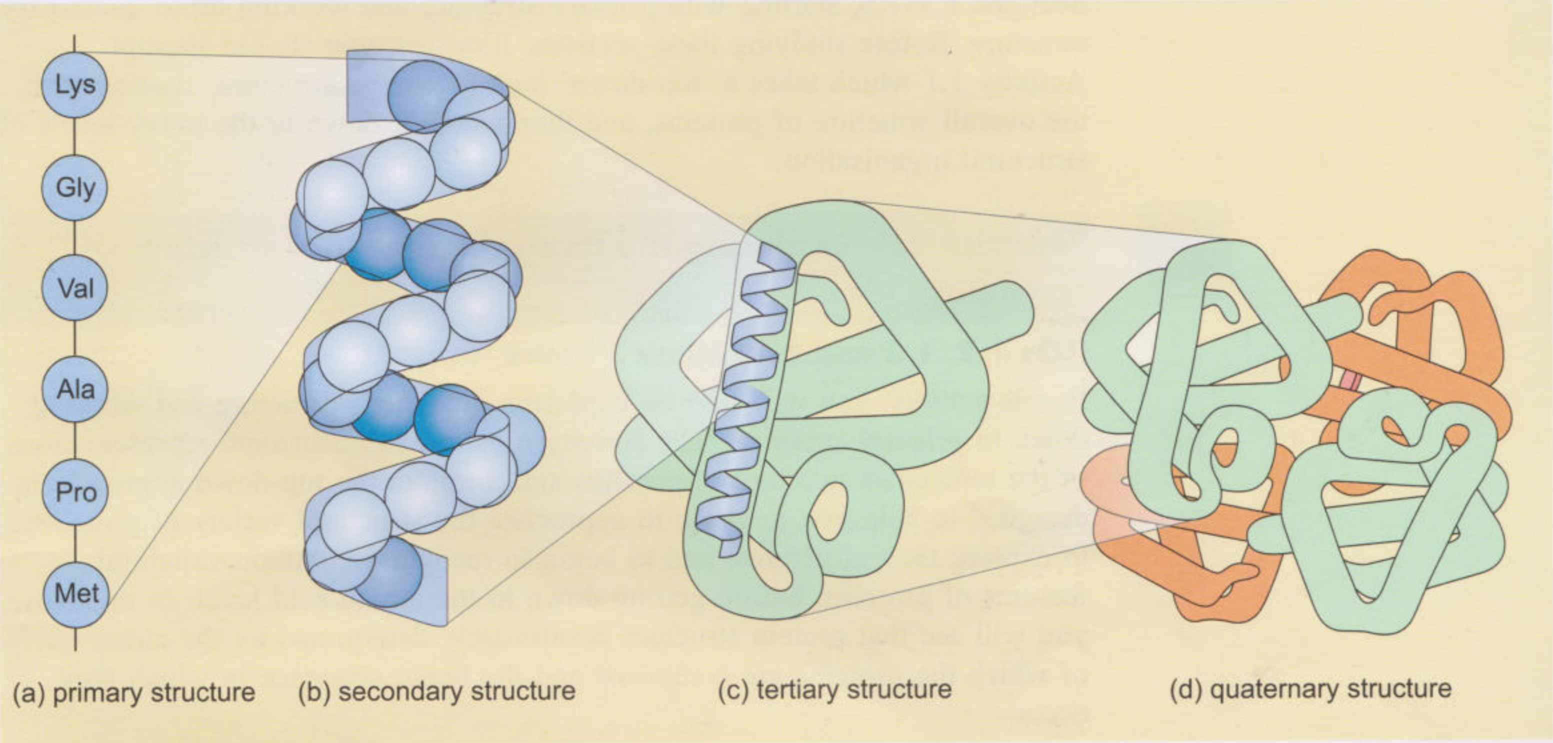


Figure 1.1 The structural hierarchy of proteins showing the four levels of organisation of protein structure: (a) primary, (b) secondary, (c) tertiary and (d) quaternary. In this example, the secondary structure represented is an α helix, which forms part of the tertiary structure of a folded polypeptide subunit. This subunit is one of four which together form the quaternary structure of this multimeric protein.

A linear chain of amino acids is known as a **polypeptide** and the linear order of the amino acids in the polypeptide, commonly referred to as the amino acid sequence, is the **primary structure** of the protein. Any particular stretch of polypeptide adopts one of a limited number of regular stable three-dimensional arrangements, which are collectively described by the term **secondary structure**. One type of secondary structure is illustrated in Figure 1.1b; here a stretch of amino acids is shown in a helical arrangement known as an α helix (alpha helix), of which you will learn more later in this chapter. A protein may contain numerous elements of secondary structure and different parts of the chain can adopt different secondary structures. The secondary structures adopted by the amino acid chain are themselves further folded and arranged to form the next level of protein structure, namely **tertiary structure**. Proteins can be composed of a single polypeptide or more than one, in which case each polypeptide is known as a **subunit** and the protein is described as 'multimeric'. The arrangement of the subunits in a multimeric protein is referred to as **quaternary structure** and is the highest level of organisation of protein structure.

A key point to understand is that the amino acid sequence determines protein structure; so for any particular type of protein, provided that conditions (such as pH) are the same, all the protein molecules will have the same structure, i.e. the polypeptides of which they are composed will all fold in the same way to adopt the same three-dimensional spatial arrangement.

The levels of structural organisation in proteins are described in more detail in Sections 1.3–1.5, starting with primary structure and working up to quaternary structure. Before studying these sections, however, you should attempt Activity 1.1 which takes a 'top-down' look at protein structure, starting with the overall structure of proteins, and then working down to the lower levels of structural organisation.

Activity 1.1 Exploring the three-dimensional structure of proteins

(LOs 1.2, 1.3 and 1.4) Allow 2 hours

In this activity, you will start by exploring quaternary structure and working down to primary structure. The activity uses three-dimensional representations of the molecular structure of proteins and, through the top-down approach, is designed to help you to begin to appreciate the structural variety of proteins, to explore their structures, and to begin to recognise common structural features of proteins, before getting down to the amino acid level. In this way, you will see that protein structure is ultimately determined by the amino acids of which the proteins are composed and the linear sequence in which they occur.

It is strongly recommended that you work through this activity at this point in your study, as it is designed to introduce some key aspects of protein structure which are developed further in the following sections of this chapter.

1.3 Proteins are built from amino acids

You will now look more closely at the amino acid building blocks of which proteins are composed, and how they are linked together to make a polypeptide chain. Twenty different amino acids are found in proteins and they have a common general chemical structure, as shown in Figure 1.2a. A basic amino group ($-NH_2$), and an acidic carboxyl group ($-COOH$), are on either side of a central carbon atom (called the α-carbon and denoted $C\alpha$). $C\alpha$ is also bonded to a hydrogen atom (H) and a variable group, referred to as the side chain or R group (represented by R in Figure 1.2a). It is the side chain that distinguishes the particular amino acid.

Molecules or groups that donate protons (such as the carboxyl group) are said to be acidic, whereas those that accept protons (such as the amino group) are said to be basic.

The side chains of the 20 amino acids that occur naturally in proteins are represented in Table 1.1. At the pH of the cytosol (~7.2 in mammalian cells), the amino and the carboxyl groups are both ionised. Thus the acidic carboxyl group loses a proton (H^+), making it negatively charged; and the basic amino group gains an H^+, making it positively charged, as shown in Figure 1.2b. Similarly, where carboxyl and amino groups occur in the amino acid side chains, they too are ionised (e.g. in the amino acids aspartate and lysine, as shown in Table 1.1).

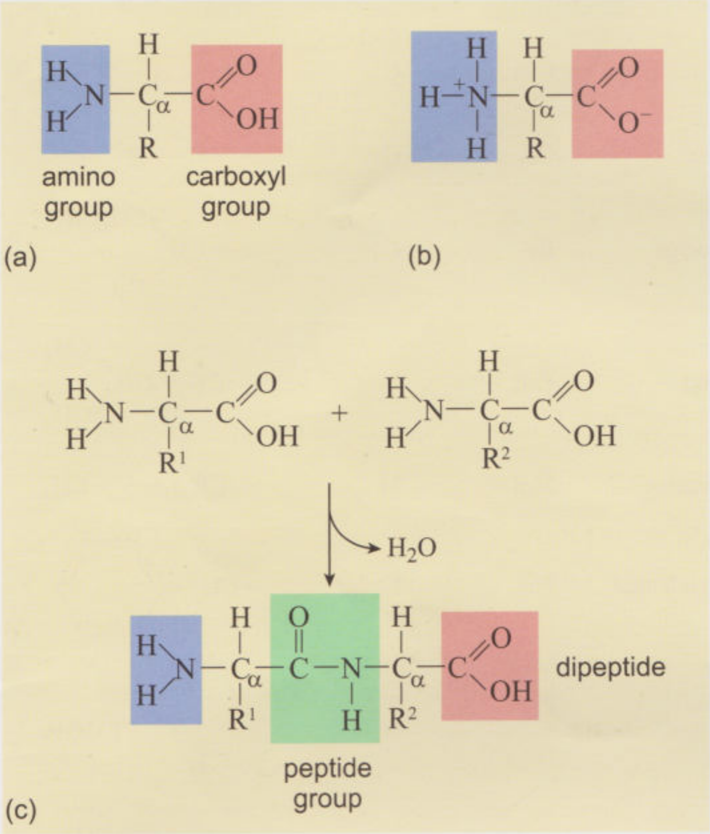
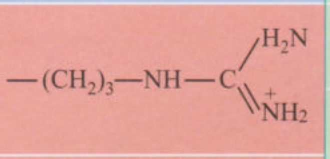
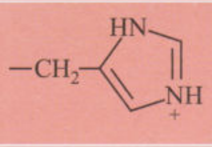
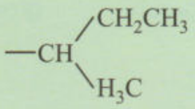
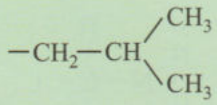
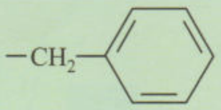
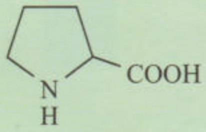
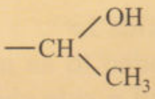
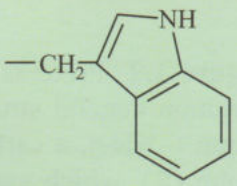
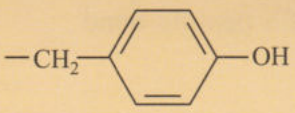
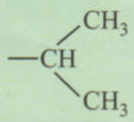


Figure 1.2 Proteins are polymers of amino acids. (a) Amino acids have a common general structure with a central carbon atom (Cα) bonded to an amino group ($–NH_2$), a carboxyl group (–COOH), a hydrogen atom and a side chain group (R), which varies between amino acids. (b) At the pH of the cytosol, the amino and carboxyl groups are ionised. (c) The formation of a peptide bond between two amino acids.

When amino acids are linked to form polypeptides during translation at the ribosome (Book 1, Section 6.6), a chemical reaction occurs between the amino group of one amino acid and the carboxyl group of another, as shown in Figure 1.2c. This is known as a condensation reaction, i.e. one in which a water molecule is eliminated as the bond forms. The resulting covalent C–N bond formed between the two amino acids is known as the **peptide bond** and the CO–NH group that forms between the neighbouring Cα atoms in the polypeptide is known as the **peptide group**. In the context of the polypeptide chain, a single amino acid unit is often described as a **residue**; short stretches of amino acid residues linked in this way are called peptides, and longer chains are polypeptides. Note that, in the polypeptide, the part of the amino acid that is common to all residues (i.e. everything apart from the side chain) forms a repeating backbone structure with the side chains (R) regularly spaced along it.

Table 1.1 The side chains of the 20 commonly occurring amino acids. Key: blue, negatively charged side chains; red, positively charged side chains; yellow, uncharged side chains; green, non-polar side chains. Proline is actually an imino acid, which does not have a free $-NH_2$ group; it does, however, form peptide links via its –NH group.

Amino acid	Symbol	One letter code	Side chain (R group)	Amino acid	Symbol	One letter code	Side chain (R group)
aspartate	Asp	D	$-CH_2-COO^-$	alanine	Ala	A	$-CH_3$
glutamate	Glu	E	$-(CH_2)_2-COO^-$	cysteine	Cys	C	$-CH_2-SH$
arginine	Arg	R		glycine	Gly	G	$-H$
histidine	His	H		isoleucine	Ile	I	
lysine	Lys	K	$-(CH_2)_4-\overset{+}{N}H_3$	leucine	Leu	L	
asparagine	Asn	N	$-CH_2-CONH_2$	methionine	Met	M	$-(CH_2)_2-S-CH_3$
glutamine	Gln	Q	$-(CH_2)_2-CONH_2$	phenylalanine	Phe	F	
serine	Ser	S	$-CH_2-OH$	proline	Pro	P	
threonine	Thr	T		tryptophan	Trp	W	
tyrosine	Tyr	Y		valine	Val	V	

- Based on Figure 1.2c, would you expect the two ends of a peptide or polypeptide to be identical?

☐ No, the two ends of a peptide or polypeptide are different. As in the dipeptide in Figure 1.2c, one end always has a free amino ($-NH_2$) group and the other has a free carboxyl (–COOH) group.

These two chemically distinct ends of the polypeptide are known as the **amino (or N-) terminus** and the **carboxy (or C-) terminus**, respectively. By convention, the amino acids in a polypeptide are numbered, and their sequence is written, from the N- to the C-terminus, reflecting the direction in which amino acids are added to growing polypeptides during protein synthesis (described in Book 1, Section 6.6).

Consider the side chains of the 20 amino acids that are found in proteins, represented in Table 1.1. You should be able to appreciate that there is quite a variety in these groups, from the single H atom in glycine to much larger and more complex side chains such as that of tryptophan. In Table 1.1, the side chains have been divided between four chemically distinct groups:

- negatively charged (acidic)
- positively charged (basic)
- uncharged **polar**, in which there is no net charge, but charge is distributed unevenly in the side chain.
- **non-polar**, in which charge is distributed evenly (or comparatively so) in the side chain.

The 'sequence' of a protein, i.e. the nature and order of amino acid building blocks of which it is composed, is of great significance for both the structure and the function of the protein. Each protein has a unique sequence and, as you will see in subsequent sections, the chemical and physical properties of the amino acid side chains influence the folding and higher-level structure of proteins and therefore the function of the protein.

Summary of Sections 1.2 and 1.3

- Proteins are composed of amino acid residues linked in linear chains, known as polypeptides.
- Amino acids have a common general structure but distinct side chains.
- The amino acid residues in a polypeptide are linked by a peptide bond formed by a condensation reaction between the amino group of one amino acid and the carboxyl group of another.
- Each protein has a unique amino acid sequence and the chemical and physical properties of the amino acid side chains determine both its structure and its function.
- Protein structure can be described in terms of four levels of organisation – primary, secondary, tertiary and quaternary.
- Primary structure is the linear sequence of amino acid residues in a polypeptide.

1.4 Secondary structure depends on hydrogen bonding

In the 1930s, Linus Pauling used X-ray diffraction (see Box 1.3, at the end of this section) to demonstrate that the peptide group (Figure 1.2c) had a rigid planar structure, i.e. the constituent atoms lie in a single geometric plane. The polypeptide chain could be seen therefore to have a backbone consisting of a series of rigid planar peptide groups, with the potential for rotation about the Cα atoms that link them. In practice, however, rotation around Cα is not entirely free because the size and chemistry (particularly the charge) of some amino acid side chains means that they cannot come too close together. Interference between chemical groups as a result of their size and position, known as **steric interference** (Box 1.2), and repulsive forces between similarly charged groups limit the range of positions available to side chains. Thus, where neighbouring residues in a polypeptide have large or similarly charged side chains, these tend to adopt a position that minimises any interference between them.

Box 1.2 Steric interference

Steric interference influences protein structure. The space occupied by individual atoms in a molecule, such as a polypeptide, is determined by the extent of their electron clouds (i.e. the space occupied by their electrons as they revolve around the nucleus). When two atoms are brought close together such that their electron clouds overlap, repulsive forces act to drive the atoms apart. This effect is known as steric interference (or steric hindrance) and it influences the conformations that a molecule can adopt. It is possible to overcome steric interference to some extent, but this requires an input of energy.

Linus Pauling was awarded the 1954 Nobel Prize for Chemistry in recognition of his contributions to understanding of protein structure.

A polypeptide will tend to fold such that it adopts the most stable **conformation**, i.e. overall shape and molecular arrangement. It was Linus Pauling who proposed, in 1951, the two most common forms of protein secondary structure: **α helices** and **β pleated sheets**. Many proteins contain both α helix and β sheet, while others contain only one of these types of secondary structure.

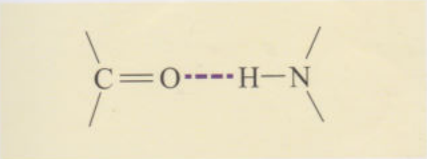


Figure 1.3 Hydrogen bond formation (purple dashed line) in protein secondary structure between the N–H group of one amino acid residue and the C=O group of another.

The arrangement of the polypeptide chain to form regions of α helix and β pleated sheet is driven by the formation of non-covalent interactions between carbonyl (C=O) and amine (N–H) groups spaced along the polypeptide backbone. These two different groups can form a hydrogen bond (Book 1, Section 1.1.1), as shown in Figure 1.3. Though individual hydrogen bonds are relatively weak and unstable (covalent bonds are anything between five and 50 times stronger), the formation of many such bonds in α helices and β pleated sheets makes these secondary-structural elements very stable.

■ In what other important biological molecule is the presence of a large number of weak hydrogen bonds structurally important?

□ DNA, where the two strands of the double helix are held together by hydrogen bonds between base pairs.

In the α helix, depicted in Figure 1.4a, the polypeptide backbone adopts a right-handed helical conformation (if you view it along its axis, the chain turns in a clockwise direction as it extends away from you) and hydrogen bonds form between the N–H of one peptide group and the C=O of the fourth peptide group along.

■ What do you notice about the distribution of the amino acid side chains in the α helix in Figure 1.4a?

- The side chains all project outwards from the helix formed by the polypeptide backbone.

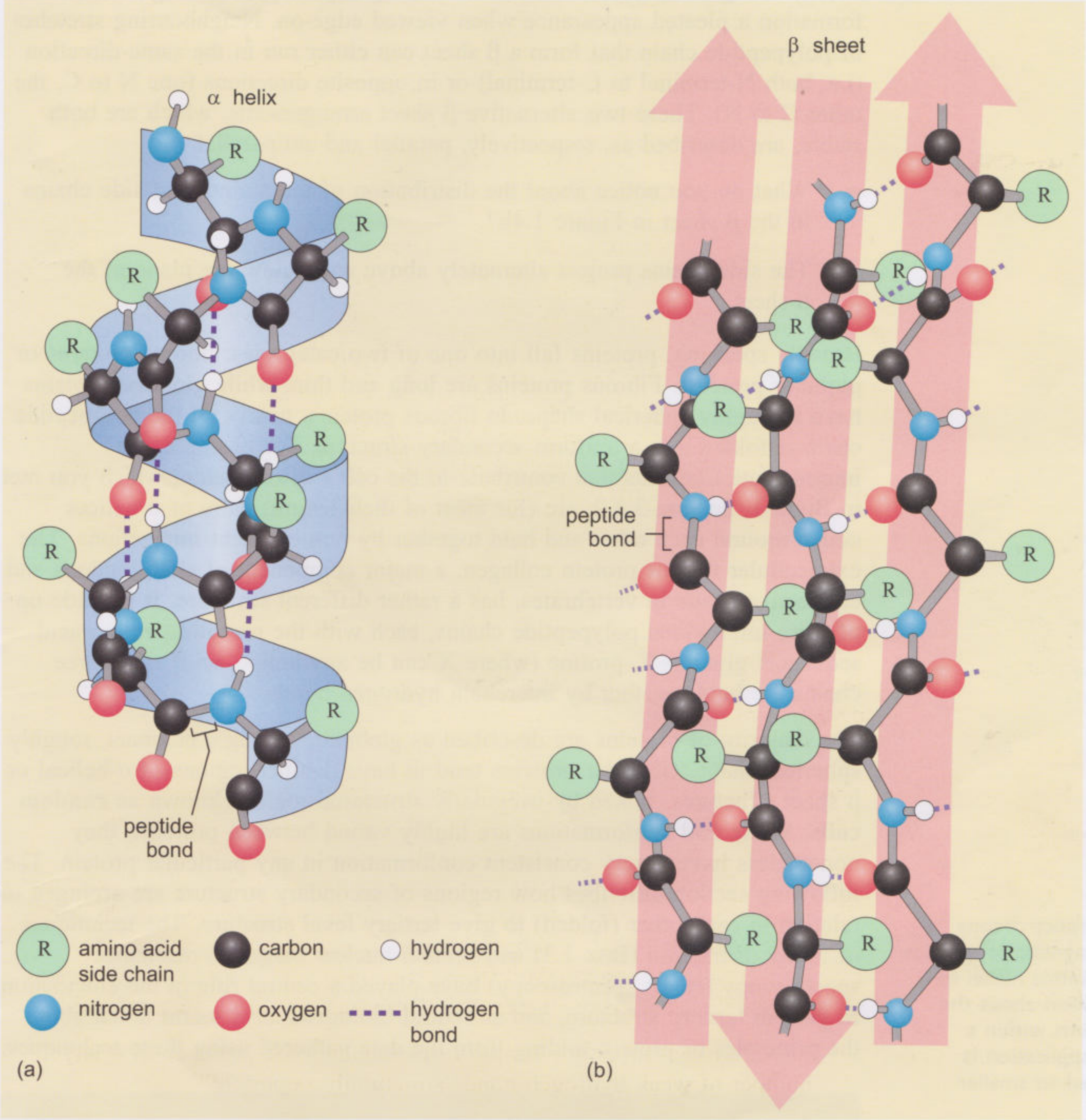


Figure 1.4 Two common secondary structure arrangements found in proteins. In these representations, the arrangement of the polypeptide backbone is highlighted by ribbons and the amino acid side chains are denoted simply by R. (a) The α helix, in which the N–H group of every peptide bond is hydrogen-bonded to the C=O group of a neighbouring peptide bond located four peptide bonds away in the same chain. (b) The β sheet; for clarity the H atoms bonded to the Cα atoms are not shown. In this example, adjacent parts of a polypeptide chain run in opposite (antiparallel) directions, as indicated by the arrow heads on the ribbons; they can also run in the same direction, in which case they are described as parallel. The different sections of polypeptide chain in a β sheet are held together by hydrogen bonds between the C=O and N–H groups of peptide bonds in the adjacent sections.

Beta sheet structures contain extended stretches of polypeptide chain arranged alongside each other with hydrogen bonding between neighbouring strands, as shown in Figure 1.4b. The strands have a zig-zag shape, which gives the sheet formation a pleated appearance when viewed edge-on. Neighbouring stretches of polypeptide chain that form a β sheet can either run in the same direction (i.e. both N-terminal to C-terminal) or in opposite directions (one N to C, the other C to N). These two alternative β sheet arrangements, which are both stable, are described as, respectively, parallel and antiparallel.

■ What do you notice about the distribution of the amino acid side chains in the β sheet in Figure 1.4b?

□ The side chains project alternately above and below the plane of the β sheet.

Broadly speaking, proteins fall into one of two categories: fibrous proteins or globular proteins. Fibrous proteins are long and thin, while globular proteins have a roughly spherical shape. In fibrous proteins, nearly all the polypeptide chain is folded into a uniform secondary structure. For example, the intermediate filaments that contribute to the cell's cytoskeleton, which you met in Book 1, Section 3.4.2, are (for most of their length) pairs of α helices coiled around each other and held together by non-covalent interactions. The extracellular fibrous protein collagen, a major component of skin, tendons and connective tissue in vertebrates, has a rather different structure. It is made up of three intertwined polypeptide chains, each with the repeating amino acid sequence: glycine–X–proline (where X can be any amino acid). The three chains are held together by interchain hydrogen bonds.

The majority of proteins are described as globular, having a compact, roughly spherical shape. Globular proteins tend to have distinct regions of α-helical or β sheet structures, linked by irregularly structured regions known as **random coils**. While coil conformations are highly varied between proteins, they nonetheless have a very consistent conformation in any particular protein. The following section describes how regions of secondary structure are arranged in relation to each other (folded) to give tertiary level structure. The techniques of X-ray diffraction (Box 1.3) and protein nuclear magnetic resonance spectroscopy (NMR spectroscopy) have played a central role in the elucidation of protein tertiary structure, and structural biologists have learnt much about the principles of protein folding from the data gathered using these techniques.

Protein NMR spectroscopy exploits the magnetic properties of particular atomic nuclei to derive information about the location of atoms within a structure. Its application is generally limited to smaller proteins.

Box 1.3 X-ray diffraction

Protein structures such as those that you have examined in Activity 1.1 have been determined experimentally, using techniques such as X-ray diffraction (Figure 1.5). The basis of this technique is that the distances between atoms in proteins are of the same order of magnitude as the wavelength of X-rays (c. 10^{-10} m). When the protein is irradiated with X-rays, the X-rays are scattered (by a process known as diffraction) as they pass through the protein. The way in which the X-rays are scattered depends on the three-dimensional positions of the atoms in the protein, and analysis of these data (i.e. the scatter) enables the shape of the protein to be determined. A basic requirement for protein structure determination using X-ray diffraction is a preparation in which the protein molecules are regularly arranged, such as they are in a crystal (when the technique is often referred to as X-ray crystallography). Protein crystals are generally obtained by adding the sample to a concentrated salt solution and allowing it to slowly precipitate. This stage has often been the stumbling block in X-ray crystallographic studies, since a number of proteins, such as those normally located in cell membranes, are difficult or impossible to crystallise.

The principal components used in X-ray crystallography are shown in Figure 1.5a, namely a source of X-rays and the protein crystal; the detector, which is connected to a computer, is not shown. A narrow beam of X-rays is fired at the crystal. Part of the beam goes straight through the crystal; the rest is diffracted (scattered) in various directions. The degree of scattering of the X-rays depends on the number of electrons in an atom: a sulfur atom, for example, scatters six times as strongly as a carbon atom. This differential scattering is important in detecting the different atoms in the final X-ray diffraction patterns obtained. Diffraction of the X-ray beams is detected as a series of spots, the X-ray diffraction pattern, in which the position and intensity of each spot contains information about the position of the atoms in the protein crystal that gave rise to it (Figure 1.5a). A computer is then used to construct three-dimensional electron-density maps from the spot intensities. In conjunction with the amino acid sequence of the protein, these maps can be used to produce a molecular model of the protein.

Interpretation of the electron-density maps requires complex mathematics. All you need to appreciate is that application of the technique of X-ray crystallography has enabled precise three-dimensional structures to be worked out for many different proteins. The protein shown in Figure 1.5 is ribulose bisphosphate carboxylase (Rubisco), an enzyme that plays a central role in carbon dioxide fixation during photosynthesis (as you will learn in Chapter 3).

At the time of writing (spring 2012), the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) holds over 64 000 different protein structures determined by X-ray crystallography. These structures are of proteins or parts of proteins from a large number of different species, and in many cases the proteins in question are associated with other proteins or molecules. In fact X-ray crystallography has also been used to study the structures of nucleic acids and protein–nucleic acid complexes. In addition, this technique has been used to help design drugs to interfere with the actions of particular proteins; an example of this, the 3C protease protein from foot and mouth disease virus, will be examined in some detail in Book 3, Chapter 3.

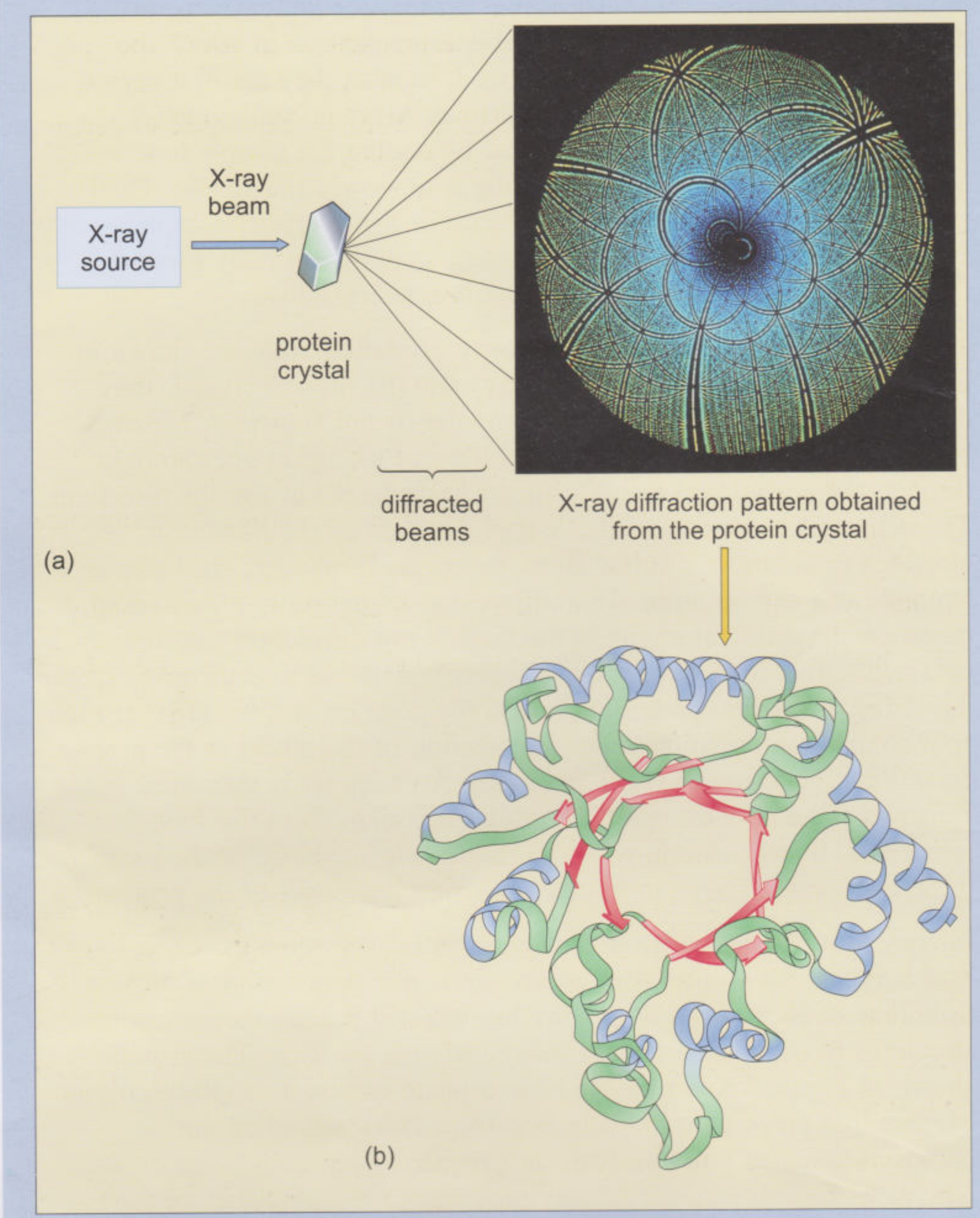


Figure 1.5 The technique of X-ray crystallography. (a) The principal components of X-ray crystallography apparatus: a source of X-rays and a protein crystal produce an X-ray diffraction pattern of the enzyme ribulose bisphosphate carboxylase (Rubisco). (b) Image of the same protein showing the major structural features (α helices in blue, β sheet in red, and random coils in green).

Summary of Section 1.4

- Secondary structure describes the regular stable arrangements of the polypeptide chain, of which the two most common are α helix and β pleated sheet.

- The formation of secondary structure is driven by the formation of hydrogen bonds between carbonyl and amino groups along the polypeptide backbone.
- X-ray diffraction is an important technique that has been used to determine the three-dimensional structure of many different proteins.

1.5 Assembling a functional protein

1.5.1 Polypeptide folding and tertiary structure

The tertiary structure of a protein is the precise and consistent overall three-dimensional arrangement of the entire polypeptide and accommodates all its secondary structures. A comparison of protein structures reveals that a number of recognisable 'motifs' or '**folds**' are common to different proteins. These are part of the tertiary structure of proteins and consist of particular arrangements of elements of secondary structure.

■ What examples of folds or motifs have you encountered in Activity 1.1?

□ The β barrel fold is a barrel-shaped arrangement of β sheet and is found in retinol-binding protein, where it serves to carry a molecule of the highly hydrophobic retinol (a form of vitamin A) in its interior. The Rossman fold is a particular arrangement of α helices and β sheet found in a variety of enzymes known as dehydrogenases, where it participates in the binding of specific molecules required for enzyme activity.

In many cases, the structure of a folded polypeptide may contain two or more physically distinct, compact substructures, known as **domains**, often linked by a flexible hinge region. Protein domains typically have distinct functions, contributing to the activity of the protein as a whole.

■ What example of a protein with clear domain structure did you encounter in Activity 1.1?

□ The enzyme Src kinase, which is involved in intracellular signalling, contains four domains; two with enzyme activity, and two that regulate the activity of the enzyme through interactions with specific ligands.

As has already been stated, a polypeptide will tend to fold such that it adopts the conformation with the highest stability. Thus the entire polypeptide chain, with its α helix, β sheet and random coil, is folded so that stability of the polypeptide as a whole is maximised. What determines the stability of a polypeptide? To understand why polypeptides fold as they do, you need to appreciate the chemical properties of the various different amino acid side chains (Table 1.1); in particular, their propensity for forming non-covalent interactions. In this respect, there are several things to note:

1 Charged groups (e.g. in lysine) tend to form ionic interactions with atoms or groups carrying the opposite charge.

2 Polar groups (e.g. in serine) and charged groups are hydrophilic (meaning 'water-loving') and hence they interact with water, which is itself a polar molecule.

3 Non-polar groups (e.g. in phenylalanine) are hydrophobic (meaning 'water-hating') and hence they preferentially interact with other non-polar molecules, via what are known as hydrophobic interactions, and avoid water.

4 A number of amino acid side chains contain atoms/groups capable of forming hydrogen bonds (e.g. between a glutamine and a serine residue).

In an aqueous environment, such as the cytosol of a cell, polypeptides destined to become globular proteins (i.e. with a roughly spherical compact overall shape adopted by soluble proteins) automatically adopt a conformation such that their hydrophobic groups are tucked away towards the interior of the molecule, out of contact with polar water molecules. The various non-covalent interactions that stabilise tertiary structure of a globular protein are represented in Figure 1.6. In the interior of the folded polypeptide, hydrophobic groups interact with each other via hydrophobic interactions. A charged group that is located in the largely hydrophobic core is typically stabilised by forming an ionic interaction with another charged group. In general, however, the hydrophilic charged or polar groups are exposed on the surface of the folded polypeptide and interact with water molecules via hydrogen bonds or ionic interactions (not shown in Figure 1.6).

Disulfide bonds (sometimes called disulfide bridges) are covalent bonds that, in some proteins, for example extracellular proteins in multicellular organisms, link different parts of a folded polypeptide chain (or two subunits in a multimeric protein, as discussed in the following section). These bonds are formed between the sulfur atoms in the side chains of two cysteine residues, and they help to stabilise the folded conformation of the protein (Figure 1.6). In most organisms, disulfide bonds are only rarely observed in intracellular proteins, though the intracellular proteins of some thermophilic bacteria, which thrive at extreme high temperatures not tolerated by other organisms (see Chapter 2 of Book 3), contain a larger than usual number of disulfide bonds, as discussed below.

With formation of disulfide bonds and the various different non-covalent interactions (hydrophobic, ionic, hydrogen bonding), the polypeptide achieves a stable three-dimensional structure. To help you understand some of the implications of this statement, it may be helpful to consider protein folding as a process by which the energy of the polypeptide is reduced. The energy of a molecule or structure is a measure of its reactivity or tendency to change, i.e. the lower its energy, the more stable it is. Stable structures are less likely to change than unstable structures and require an input of energy to disrupt them. In the case of proteins, although individual non-covalent interactions are relatively very weak when compared with a covalent bond (i.e. much less energy is required to disrupt them), the number of such interactions in a folded polypeptide is very large and the stability of the folded polypeptide can be attributed to their large number. Importantly, it is the final conformation of the polypeptide *as a whole* that is important and often a less favourable position may be tolerated for individual residues if the folded polypeptide ultimately has a stable conformation.

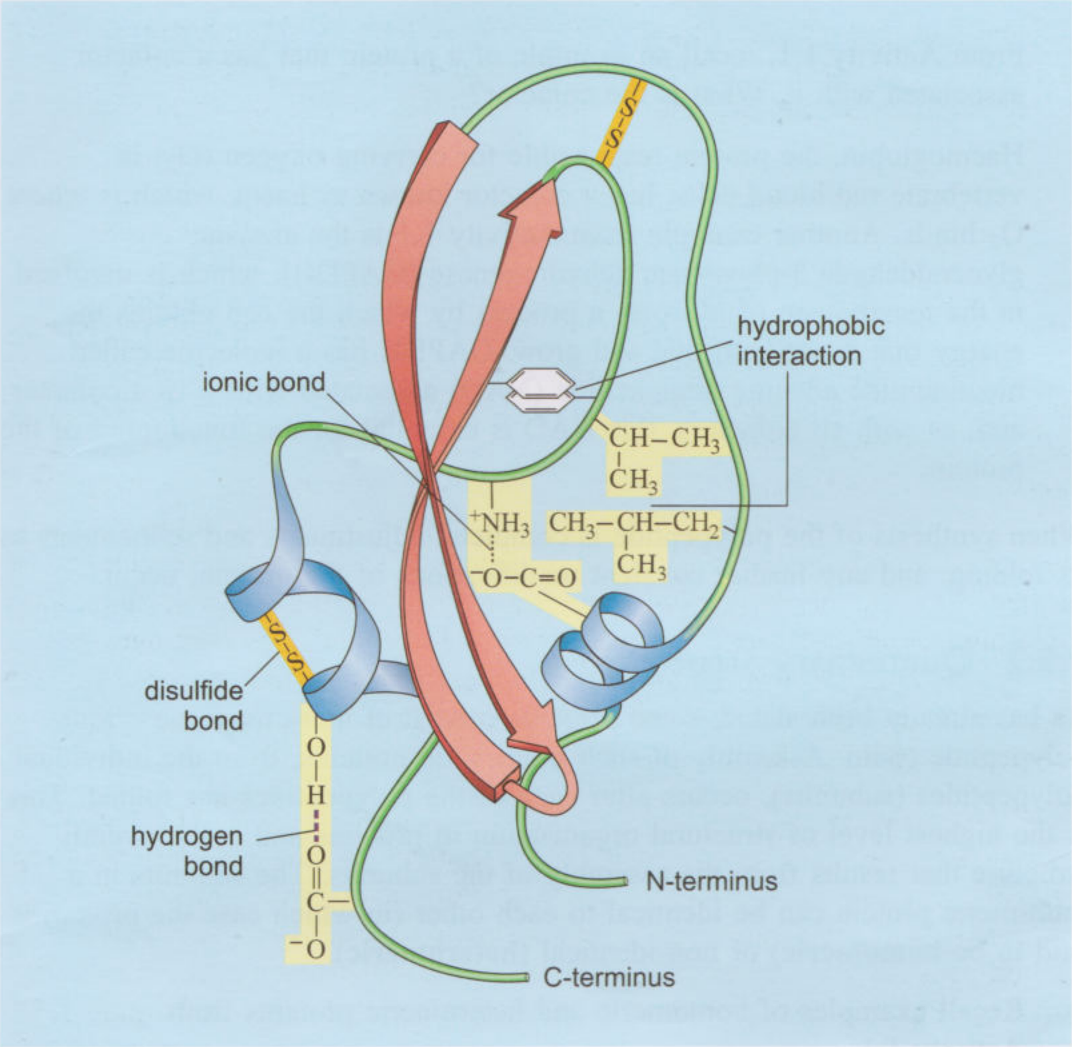


Figure 1.6 Tertiary structure of a small stylised globular protein stabilised by several types of weak interaction between side chains and by covalent disulfide bonds. (Note that the interactions are not shown to scale with the 'backbone' of the polypeptide chain; in reality, the 'backbone' is much larger than the individual side chain features shown.)

■ In thermophilic bacteria, mentioned above, the intracellular proteins have a larger than usual number of disulfide bonds. How would this feature help the bacteria to survive extreme high temperatures?

□ Disruption of protein structure would compromise the function of affected proteins and hence the survival of the organism. A large amount of energy is required to break covalent bonds; hence the larger number of disulfide bonds help to stabilise the protein structures and prevent the disruption of the proteins by the extreme high temperature.

Polypeptides start to adopt elements of secondary structure and to fold even as they are being synthesised. Initial folding is rapid (taking only a few seconds). Some proteins have associated non-protein molecules, known as **cofactors**, which are essential to their function, and such cofactors will associate with the polypeptide as it folds. Cofactors are discussed further in Section 1.8.3.

- ■ From Activity 1.1, recall an example of a protein that has a cofactor associated with it. What is the cofactor?

- □ Haemoglobin, the protein responsible for carrying oxygen (O_2) in vertebrate red blood cells, has a cofactor known as haem, which is where O_2 binds. Another example from Activity 1.1 is the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which is involved in the metabolism of glucose, a process by which the cell obtains the energy that it needs to live and grow. GAPDH has a molecule called nicotinamide adenine dinucleotide (NAD) associated with it as a cofactor and, as with all cofactors, this NAD is essential for the functioning of the protein.

When synthesis of the polypeptide is complete, adjustments and refinements to its folding, and any further covalent modifications of the protein, occur.

1.5.2 Quaternary structure

As has already been stated, some proteins consist of more than one polypeptide chain. Assembly of such multimeric proteins, from the individual polypeptides (subunits), occurs after each of the polypeptides has folded. This is the highest level of structural organisation in proteins and is the overall structure that results from the assembly of the subunits. The subunits in a multimeric protein can be identical to each other (in which case the protein is said to be **homomeric**) or non-identical (**heteromeric**).

- ■ Recall examples of homomeric and heteromeric proteins from Activity 1.1.

- □ Haemoglobin is heteromeric, having two each of two different subunits (four subunits in total). The cytokine interleukin-8 is homomeric, having two identical subunits.

As with tertiary structure, quaternary structure depends on non-covalent interactions; and here the interactions are between the surfaces of folded subunits. In addition, in some cases, covalent disulfide bonds (described in the previous section), between cysteine residues in two different subunits, help to stabilise the quaternary structure of a protein, and are commonly found in secreted or extracellular proteins.

- ■ From Activity 1.1, can you recall a secreted protein that contains disulfide bonds between as well as within subunits?

- □ The hormone insulin, which is secreted by a specific cell type in the pancreas in vertebrates, contains three disulfide bonds. Two of the bonds link the two polypeptide chains and one is within one of the chains (shown in Figure 1.8).

The mature insulin protein, with its two polypeptide chains, is actually produced by proteolysis of a single polypeptide chain (called proinsulin; see Section 1.6.2).

1.5.3 Chaperones help polypeptides to fold

It has been shown that, in a suitable chemical environment, the polypeptide chains of many small single-domain proteins will spontaneously fold *in vitro* (i.e. in a test tube), adopting the conformation that they have in the natural cellular environment. In such cases, the primary structure of the polypeptide is sufficient to direct the folding process. Larger proteins, or those with more than one domain, do not do this, however; and *in vivo* (i.e. in a living organism), many polypeptides require specialised proteins called **chaperones** to help them to fold.

Chaperones help polypeptides to fold by preventing inappropriate interactions. They can also help to recover and refold polypeptides that have 'misfolded', i.e. that have adopted incorrect or aberrant structures. More than one chaperone protein may be required to facilitate folding, with the polypeptide being passed between them in sequence. It seems that the chaperones do not direct the folding as such, but they help to prevent the polypeptide from aggregating with other molecules (including other molecules of the polypeptide itself). Certain chaperone proteins consist of 14 subunits arranged as two doughnut-shaped rings sitting on top of each other (one such ring is shown in Figure 1.7). The chaperoned protein sits inside the hole formed by these rings, where it is protected from aggregation while fine adjustment to its folding occurs.

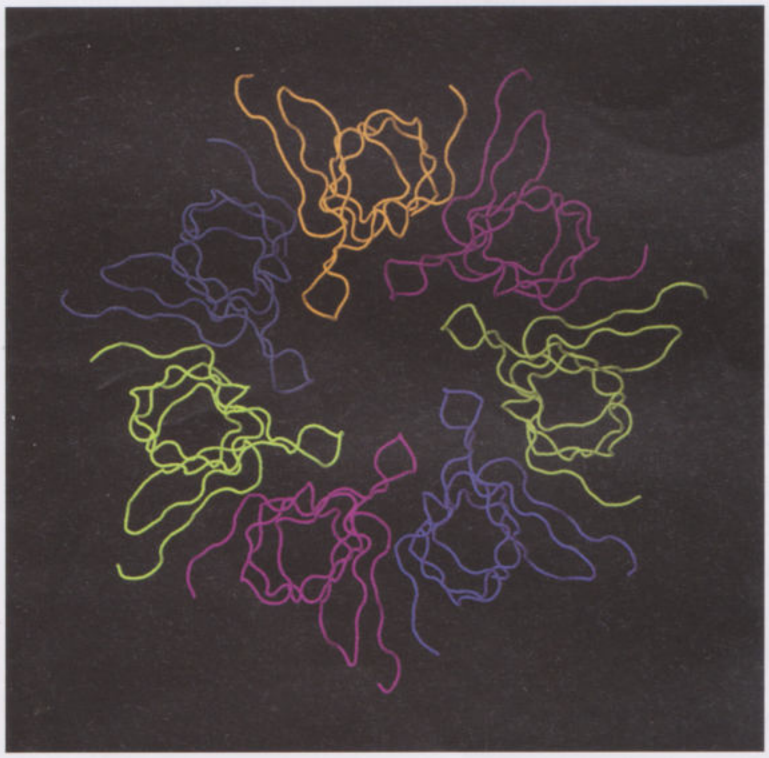


Figure 1.7 Part of a chaperone protein from *Mycobacterium leprae* (heat shock chaperonin-10; Protein Data Bank entry 1lep). Seven subunits are arranged in a ring, as depicted here (showing the backbone only), and two such rings sit on top of each other in the complete structure. The hole at the centre of this large multimeric protein is where the chaperoned protein sits as it folds.

1.5.4 The proteasome breaks down misfolded and unwanted proteins

Despite the presence of chaperones, misfolded proteins do occur and recognition and degradation of these is essential for the health of the cell. It is thought that up to one-third of newly synthesised polypeptides are degraded rapidly by a family of proteolytic (i.e. protein-digesting) enzymes known as **proteases**, which catalyse the cleavage of peptide bonds. In eukaryotic cells, most proteins are broken down in the cytosol by large complexes of proteolytic enzymes called **proteasomes**.

The proteasome breaks down not only misfolded proteins, but also those proteins that are no longer required by the cell. They are therefore an important component of the cellular systems that help to regulate the levels of proteins in the cell, along with control at the level of gene transcription and translation discussed in Chapter 6 of Book 1. Proteins are prepared for degradation by the covalent attachment to a protein known as ubiquitin, a process catalysed by specialised enzymes. Attachment of ubiquitin in this way targets the protein in question for degradation by the proteasome.

1.5.5 Protein misfolding and disease

Aberrant misfolded proteins at the very least may not be able to perform their particular function, and at worst may be directly damaging to the cell and/or tissue in which they are produced. Despite the activity of chaperones and proteasomes or other protective mechanisms, cellular functions may still be compromised by the formation of extracellular or intracellular aggregates of misfolded proteins. This is in fact the basis of a number of conditions that affect humans, including cystic fibrosis and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease. (Huntington's disease is discussed in some detail in Chapter 3 of Book 3.)

Cystic fibrosis is most commonly caused by deletion of a short stretch (only three nucleotides) of a gene known as *CFTR*. This gene encodes a protein known as the **c**ystic **f**ibrosis **t**ransmembrane conductance **r**egulator, which is involved in the transport of chloride ions across cell membranes. The three-nucleotide deletion in the *CFTR* gene results in the omission of a single amino acid residue from the protein, which in turn causes a delay in the folding of the polypeptide. The delay is long enough for the mutant CFTR protein to be targeted for removal as an abnormal protein. Even if the folded mutant protein could potentially function normally, the efficiency of the cellular housekeeping is such that it does not have the opportunity to do so and the symptoms of cystic fibrosis result from a deficiency in the CFTR protein.

Alzheimer's disease, which is the most common neurodegenerative disease, is thought to be caused by accumulation of abnormally folded beta-amyloid (Aβ) and tau proteins in the brain. Aβ is produced as a result of proteolysis of a protein called amyloid precursor protein (APP), which is located in neuronal cell membranes. The Aβ fragment forms aggregates that accumulate in the extracellular matrix of the central nervous system and which are toxic to neurons. Aggregation of tau protein, which normally helps to stabilise the microtubule network within the neuron, results in formation of neurofibrillary tangles inside neurons.

■ What is the function of microtubules?

□ They are components of the cytoskeleton and help to maintain cell shape as well as being important for intracellular movement such as movement of cell organelles from one part of the cell to another, and the segregation of chromosomes into the daughter cells during cell division (Section 3.4.2 of Book 1).

The neurofibrillary tangles that form in central nervous system neurons in Alzheimer's disease cause the microtubule network of affected neurons to be destabilised, and, as a result, intracellular transport in the neuron is compromised.

Variant Creutzfeld–Jakob disease (vCJD), the human form of bovine spongiform encephalopathy ('mad cow disease'), is an example of a prion disorder. In such conditions, a normal cellular prion protein adopts a misfolded pathological conformation. The misfolded prion protein causes other correctly folded prion protein molecules to adopt the same misfolded conformation. The process by which this occurs is beyond the scope of this text, and indeed is still a topic of intense research. As in Alzheimer's disease, the misfolded protein molecules assemble into damaging aggregates which cause neurodegeneration.

Why do misfolded proteins tend to aggregate as they do in the diseases described above? One theory is that the misfolded protein molecules self-associate because they have exposed hydrophobic regions.

■ How would you predict that two molecules of misfolded protein with exposed hydrophobic regions would interact in an aqueous environment?

□ They would interact via their hydrophobic regions, thereby excluding water molecules.

Thus, the aggregated form is more stable than the free protein and may be further stabilised by the formation of intermolecular hydrogen bonds (i.e. between protein molecules). Interactions between many molecules in this way lead to the formation of large microscopically visible insoluble aggregates that are typical of many protein misfolding disorders. Aggregates seem to escape degradation by the usual cellular mechanisms that remove damaged and misfolded proteins. In some cases, this may be because they form outside the cell (after the protein has been secreted). Where aggregates form inside the cell, there is some evidence that the mechanisms that normally protect the cell by removing aberrant proteins are compromised.

Summary of Section 1.5

- The tertiary structure of a protein is the overall three-dimensional arrangement or folding of the polypeptide chain and its secondary structures. Folding of the polypeptide to achieve this tertiary structure increases its stability by accommodating the various different amino acid side chains in an arrangement in which they can form appropriate non-covalent interactions. In some proteins, disulfide bonds formed between the sulfur atoms of two cysteine residues in different parts of a folded polypeptide chain help to stabilise its folded conformation.

- Certain folds or motifs are common to different proteins and many proteins have compact substructures known as domains within the folded polypeptide. Protein domains often have defined functions within the protein as a whole.
- During the folding process, some proteins associate with cofactors, non-protein molecules that are essential to the function of the protein.
- Quaternary structure only exists in proteins that contain more than one subunit (polypeptide) and refers to the association of the individual folded subunits to form a multimeric protein. In some cases, disulfide bonds form between subunits of multimeric proteins.
- Many proteins require chaperones to help them fold correctly. When proteins misfold, they are targeted for degradation by attaching ubiquitin to them. This identifies the misfolded protein for destruction by proteasomes.
- Misfolding of proteins occurs in a number of medical conditions that affect humans, including cystic fibrosis, and neurodegenerative diseases such as Alzheimer's disease.

1.6 Post-translational modification of proteins

Proteins that carry out their role in the cytosol are fully functional as soon as the polypeptide is released from the ribosome and has become correctly folded. Other proteins of a eukaryotic cell are synthesised on ribosomes attached to the cytosolic surface of the endoplasmic reticulum (ER), and those that are intended for export out of the cells or for incorporation into membranes pass into the lumen of the ER, where they undergo **post-translational modification**. (The targeting of newly synthesised proteins to their appropriate destinations inside or outside of the cell was discussed in Section 3.4 of Book 1.)

1.6.1 Covalent modification of proteins

Some post-translational modifications involve the addition of particular chemical groups, such as sugars or lipids. The addition of short sugar chains to specific sites on the folded protein is known as protein **glycosylation**. The sugars are covalently attached to the protein by reacting with the $-NH_2$ or $-OH$ groups of particular amino acid side chains, to form a **glycoprotein**. Many glycoproteins are incorporated into the cell membrane (see Chapter 2) and have important roles in intercellular interactions; others are secreted by the cell and function outside it. Glycosylation of proteins may offer them some protection against proteases, by restricting access of these enzymes to the protein component.

The covalent attachment of one or more lipid groups to a protein (protein **lipidation**) results in the formation of what is known as a *lipid-linked protein*. The lipid component of such proteins serves to anchor them in membranes, and the nature of the lipid group that is attached affects which side of a particular membrane a protein will locate to: for example, the cell membrane, the endoplasmic reticulum or the nuclear membrane of eukaryotic cells. You will learn more about lipid-linked proteins in the following chapter.

1.6.2 Activation of proteins by proteolytic cleavage

Apart from covalent modification of proteins by the addition of chemical groups, some proteins may be post-translationally modified by the hydrolysis of specific peptide bonds. This is the case for certain secreted enzymes called **zymogens**, which are secreted in an inactive form but are converted into active proteins outside the cell by proteolytic cleavage. Examples include the digestive enzymes pepsin and trypsin, whose secretion as zymogens ensures that the cells secreting them are not damaged (by digestion) in the process.

Another example of a protein that is converted into its active form by proteolytic cleavage is the hormone insulin, which is synthesised as proinsulin. The proinsulin polypeptide has a tertiary structure that is stabilised by disulfide bonds (Figure 1.8). Proteolysis removes a section of the proinsulin polypeptide chain, known as the connecting peptide, leaving two polypeptide chains joined by two disulfide bonds.

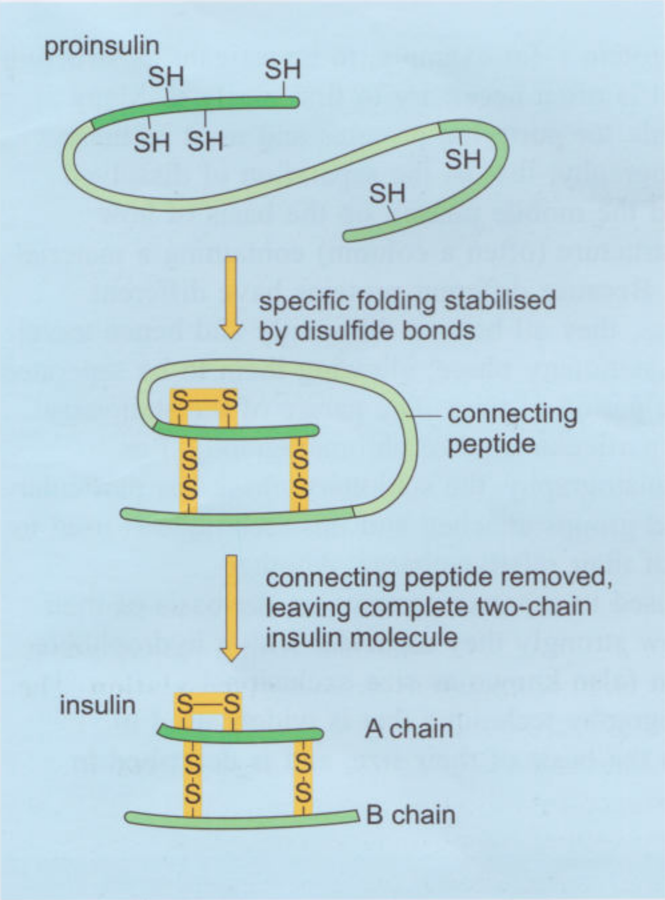

proinsulin
SH
SH
SH SH
SH
SH
specific folding stabilised by disulfide bonds
S—S
S S
S S
connecting peptide
connecting peptide removed, leaving complete two-chain insulin molecule
insulin
S—S
S S
S S
A chain
B chain

Figure 1.8 Insulin is derived from proinsulin by proteolytic cleavage. Proinsulin is a single large polypeptide, which folds to form a tertiary structure that is stabilised by disulfide bonds. Cleavage of proinsulin by proteolysis then removes a connecting peptide to form the biologically active molecule, insulin.

Summary of Section 1.6

- Some proteins are modified post-translationally by covalent modification or proteolytic cleavage.
- Irreversible covalent modifications include glycosylation, in which sugar chains are added to the protein, and lipidation, in which one or more lipid groups are attached to the protein. In both cases, groups are added at specific sites on the protein. Glycoproteins are either secreted or incorporated in the cell membrane. Lipid-linked proteins are anchored in membranes via their lipid component.
- Zymogens are inactive forms of certain enzymes that are converted into active proteins outside the cell by proteolytic cleavage.
- Mature active insulin is produced by proteolytic cleavage of proinsulin.

1.7 Purifying and analysing proteins

In Section 3.2.2 of Book 1, you learnt about how cells can be homogenised and fractionated to study their components. The proteins in cell homogenates or subcellular fractions can be identified or analysed using a variety of techniques.

In order to study a particular protein – for example, to investigate its structure or to investigate its activity – it is often necessary to first purify it. Many techniques are currently available for purifying proteins and most of these involve some form of chromatography; that is, the separation of dissolved substances in a mixture (termed the mobile phase), on the basis of how quickly they travel through a structure (often a column) containing a material known as the stationary phase. Because different proteins have different physical and chemical properties, they all behave differently and hence travel at different speeds through the stationary phase, allowing them to be separated from each other over a suitable period of time. The nature of the stationary phase varies depending on the particular kind of chromatography. For example, in ion exchange chromatography, the stationary phase has particular positively or negatively charged groups attached and this technique is used to separate proteins on the basis of their relative charge. Another chromatographic technique is used to separate proteins on the basis of their relative hydrophobicity (i.e. how strongly they associate with a hydrophobic stationary phase). **Gel filtration** (also known as size exclusion chromatography) is a chromatography technique that is widely used to separate and purify proteins on the basis of their size, and is described in Box 1.4.

Box 1.4 Gel filtration

Gel filtration involves the use of a hollow plastic or glass column filled with a polysaccharide gel. The gel contains small porous beads, each consisting of a three-dimensional water-filled network which acts as a molecular sieve: small molecules enter and linger in the pores, so their passage down the column is retarded; larger molecules, unable to enter the pores, pass more quickly down the column between the beads. When a mixture of different-sized proteins is added to the column, the molecules are separated on the basis of their size difference (Figure 1.9). The largest molecules are washed through quite quickly (washing through is termed 'elution'). Smaller molecules can enter some of the larger pores and so take longer to pass through the column, and molecules below a certain size can enter even the smallest pores, and so take longer still to pass through. The proteins can be collected in separate fractions as they are eluted from the column. Different gels can be used with different pore sizes, so passage down two or three columns can improve separation. Different-sized proteins are therefore separated on the basis of size, and an elution profile is produced (Figure 1.10).

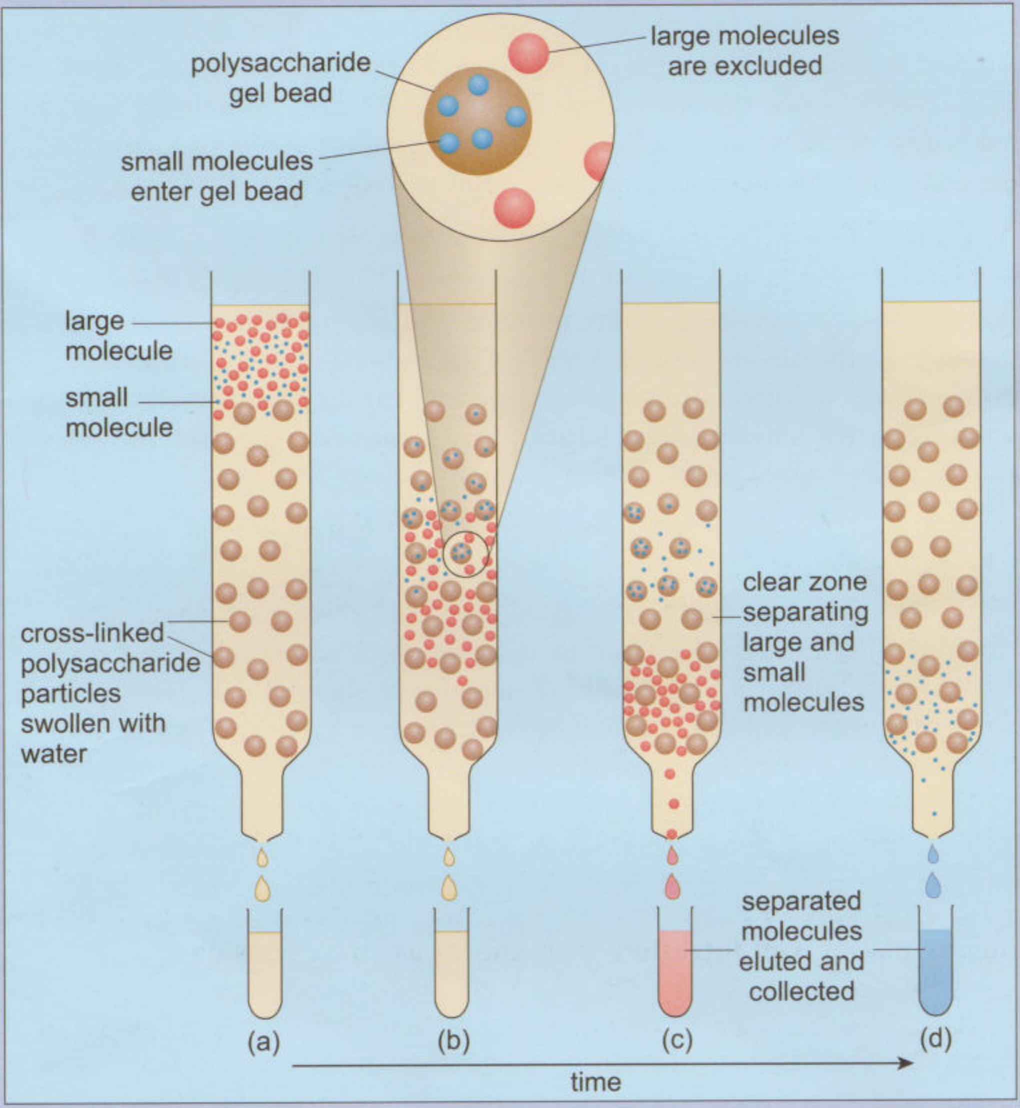

polysaccharide gel bead
large molecules are excluded
small molecules enter gel bead
large molecule
small molecule
cross-linked polysaccharide particles swollen with water
clear zone separating large and small molecules
separated molecules eluted and collected
(a)
(b)
(c)
(d)
time

Figure 1.9 Gel filtration. (a) A solution containing large and small protein molecules is applied to the gel column. (b) Small protein molecules take longer to pass through the column than do larger proteins (enlargement shows the interaction of proteins with the beads). Large proteins are eluted first (c), followed by smaller ones (d).

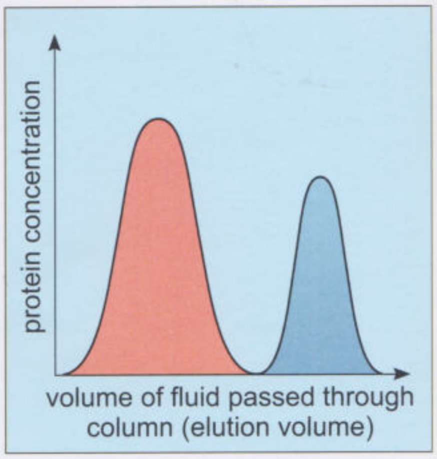


Figure 1.10 An elution profile showing the separation of two different-sized protein molecules.

While gel filtration can be used in the preparation of pure proteins, it can also be used on a smaller scale for analysis: for example, to determine the purity of a protein sample, to measure the concentration of a particular protein in a mixture, or to estimate the M_r of a protein. **SDS polyacrylamide gel electrophoresis** (abbreviated to **SDS–PAGE**) and **immunoblotting** are further techniques that are widely used to analyse proteins in a mixture (e.g. a cell homogenate) and are described in Box 1.5.

Box 1.5 SDS–PAGE and immunoblotting

SDS–PAGE

Electrophoresis encompasses a variety of different techniques, all of which entail the application of an electric field to a sample to separate charged molecules. The molecules are separated as they move through a support medium (often a gel) in response to the applied electric field. SDS–PAGE is one particular type of electrophoresis, commonly used for analysing proteins, in which the rate of movement of the proteins depends only on their size, i.e. their relative molecular mass (M_r).

A solution containing the proteins is treated with a detergent called sodium dodecyl sulfate (SDS) and is heated. The negatively charged dodecyl sulfate ions bind to the proteins and, with heating, cause them to denature (unfold). The dodecyl sulfate ions give the unfolded polypeptides a uniform negative charge that masks any intrinsic charge due to charged amino acid residues. This means that the proteins carry a charge that is approximately proportional to their M_r.

The apparatus used for SDS–PAGE is shown in Figure 1.11. The samples, to which a coloured dye of low M_r is added, are loaded into small slots ('wells') in a thin slab of polyacrylamide gel (which also contains SDS to keep the proteins denatured). When an electric field is applied, the buffer, the dye and the negatively charged denatured protein molecules enter and move down the gel towards the positive electrode. As the gel is a complex three-dimensional network, the migrating protein molecules have to pass through narrow, tortuous passages in the gel as they are drawn towards the positive electrode. Smaller molecules pass through the gel more easily than do large molecules, and hence in a given time, small proteins move further into the gel than do large proteins. The migration rate for the proteins can thus be said to be inversely related to their M_r.

Electrophoresis is discontinued by switching off the electric field when it is judged that resolution is sufficient (generally from noting how far the low M_r dye has travelled). The separated proteins can then be visualised by staining with a dye that binds to proteins. If resolution has been effective, proteins can be seen as a ladder of discrete bands in the gel (Figure 1.12). The polyacrylamide gel used in SDS–PAGE is prepared by polymerisation of acrylamide monomers and the concentration of acrylamide is chosen to optimise the separation of the proteins under investigation, with higher concentrations being used for analysing smaller proteins.

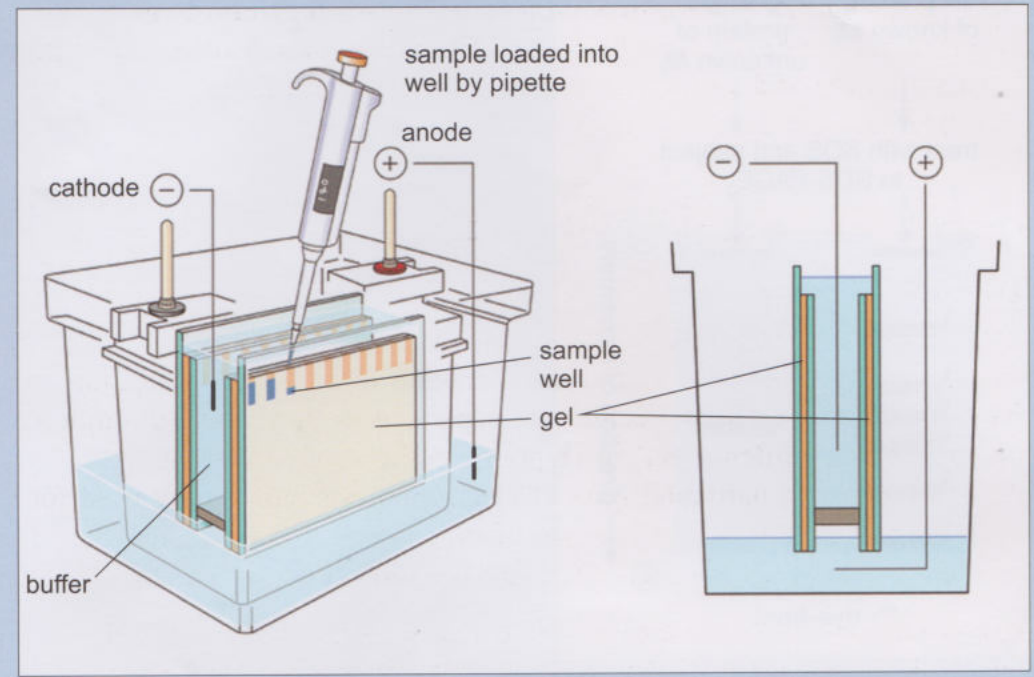


Figure 1.11 Schematic diagram of the vertical slab gel electrophoresis apparatus, here showing two gels, each capable of running 10 samples simultaneously. During its preparation, the liquid gel has been allowed to harden with an appropriately shaped mould in place to make the wells for the samples. Protein samples are pretreated with SDS and contain a dye. During electrophoresis, an electric field is applied and the negatively charged protein molecules move towards the positive electrode (the anode). After electrophoresis, the proteins can be stained, revealing them as discrete bands. The region of the gel in which the components of one sample can move is called a lane; thus, each of the two gels shown here has ten lanes.

SDS–PAGE is a powerful tool in protein analysis. It can be used to separate all types of protein; even membrane proteins and protein components of the cytoskeleton, which are normally insoluble in water, are solubilised by treatment with SDS. Since the method separates on the basis of size, the approximate M_r of a protein may be deduced by comparing its migration in the gel with the migration of standard proteins of known M_r (Figure 1.12). The distance travelled by each protein is measured relative to the distance travelled by the dye-front; the ratio of the two distances is called the relative electrophoretic mobility, R_f. The M_r values of the six proteins in lane 1 are plotted against their R_f values in Figure 1.13. From Figure 1.13, it can be seen that the M_r of protein X = 3×10^4 = 30 000. Note that this value for M_r provided by SDS–PAGE is only approximate and in practice a more accurate value is obtained using other techniques.

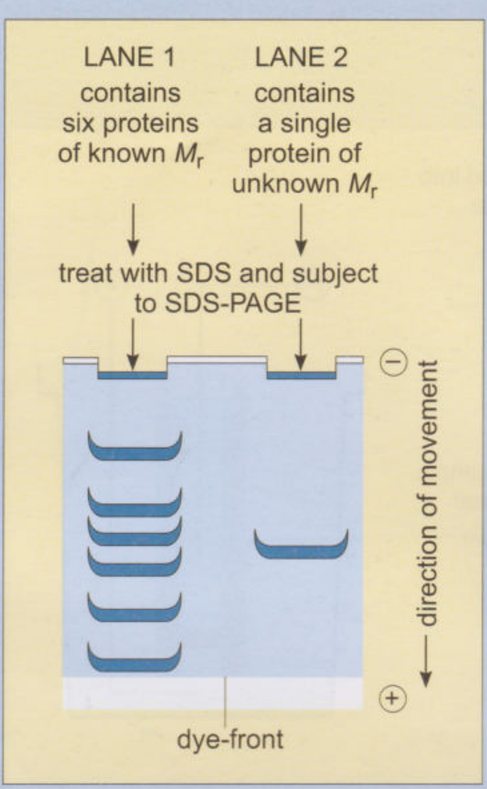

LANE 1
contains six proteins of known M_r
LANE 2
contains a single protein of unknown M_r
treat with SDS and subject to SDS-PAGE
−
+
direction of movement
dye-front

Figure 1.12 Schematic representation of the gel pattern produced in two lanes of an SDS–PAGE experiment. The well for lane 1 contained a mixture of proteins of known M_r. By comparison with these standards, the M_r of the protein in lane 2 can be estimated.

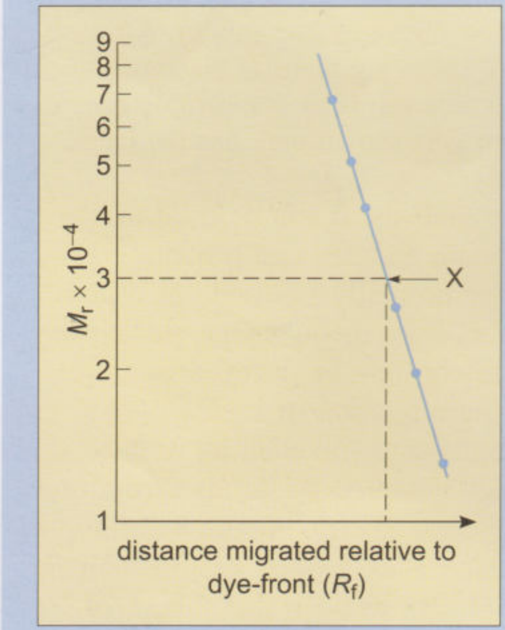


Figure 1.13 Determination of M_r for an unknown protein X by comparing its mobility with that of six standard proteins of known M_r. Note that the M_r values of the proteins have been multiplied by 10^{-4} to obtain the numerical values indicated on the y-axis. Thus the value of 3 on the y-axis for the unknown protein X corresponds to an M_r of 3×10^4.

Though SDS–PAGE is only used for *analysis* of proteins and is not suitable for the purification of large quantities of material, it is a very valuable technique when it comes to analysing composition at different stages in the purification of proteins. In such cases, the protein preparation is subjected to SDS–PAGE, the gel is stained and the pattern of bands examined. Each band corresponds to an individual protein molecule. If there are several bands in a single lane, then the preparation still contains a mixture of proteins. A single band could indicate purity.

- ■ What is the problem with the latter interpretation?
- □ Two different proteins of the same size would appear as a single band.

Figure 1.14 is a photo of a real gel, showing successive stages in the purification of a protein complex, indicating approximate values of M_r.

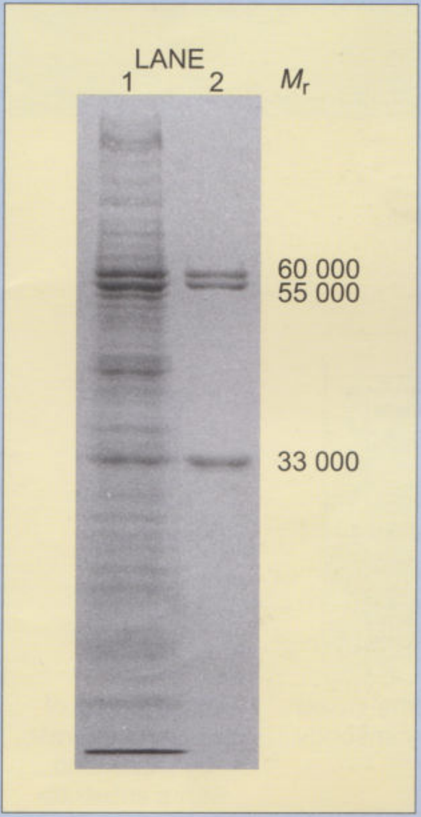


Figure 1.14 Photo of a real gel showing two stages in the purification of a protein complex. Lane 1 shows crude extract from the prokaryote *Azotobacter chroococcum*. Lane 2 shows a preparation consisting of three protein bands isolated from the crude extract.

In SDS–PAGE, unless individual protein subunits are linked by a disulfide bond, they all separate and travel as discrete bands. Disulfide bonds can however be broken by treating the sample with a suitable chemical (such as β-mercaptoethanol or dithiothreitol) that breaks the bond between the two sulfurs and restores the sulfhydryl groups. This is a reduction reaction (see Box 3.1 in Chapter 3). Such treatment separates subunits linked by disulfide bonds prior to analysis by SDS–PAGE. In this way, it is possible to determine the subunit composition of a protein.

Immunoblotting

Although SDS–PAGE tells us a lot about the size and composition of a protein, it cannot tell us much about its identity. Immunoblotting (also known as western blotting) is used to identify individual proteins in a mixture of proteins that have been separated by SDS–PAGE. The separated proteins in the polyacrylamide gel are transferred (blotted) onto a support material called nitrocellulose (often referred to as a filter) by applying an electric field across the gel. Protein-specific antibodies (Book 1, Box 2.4) are then used to identify individual proteins on the filter. Usually this is done in two stages. A primary antibody recognises the protein of interest and then a secondary antibody, which recognises the primary antibody and which carries a label to permit its visualisation, is applied. Detection of this secondary antibody reveals the location of the protein of interest among the various proteins that were separated in the electrophoresis stage (Figure 1.15).

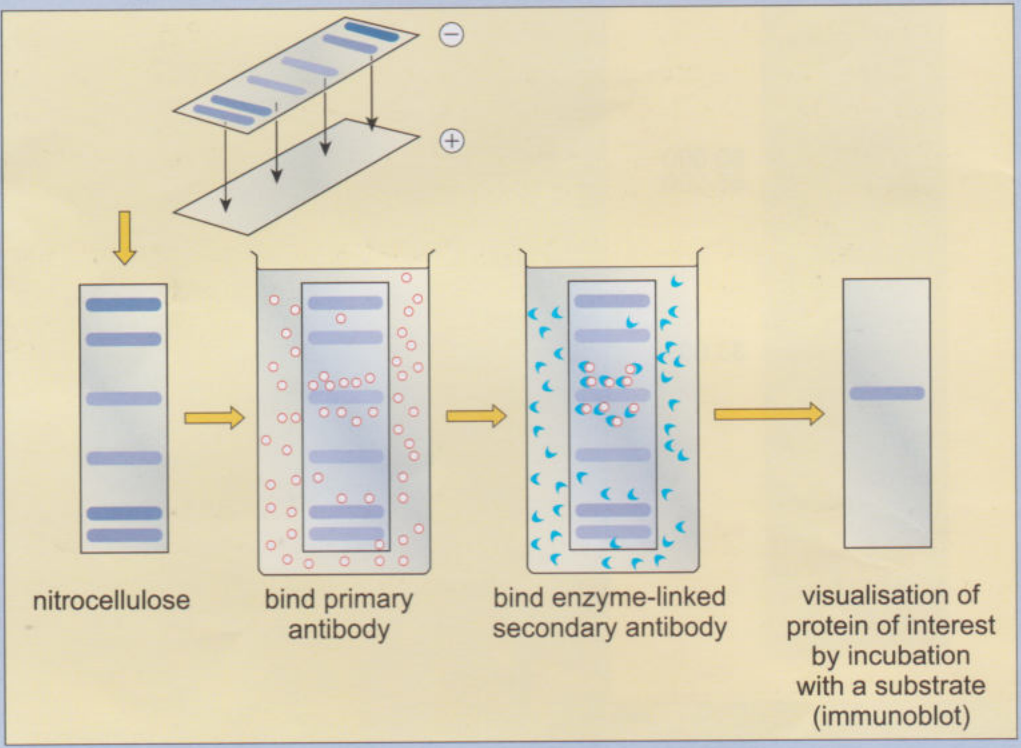

⊖
⊕
nitrocellulose
bind primary antibody
bind enzyme-linked secondary antibody
visualisation of protein of interest by incubation with a substrate (immunoblot)

Figure 1.15 Following SDS–PAGE, proteins in the gel can be transferred to a nitrocellulose filter and specific proteins identified by immunoblotting. This requires an antibody (the primary antibody) that specifically recognises the protein of interest. A secondary antibody that recognises the primary antibody, and to which is attached some kind of label (usually an enzyme such as peroxidase), is then applied. The presence of the secondary antibody, and hence the protein of interest, is detected by incubation with a substrate for the enzyme. The product of the enzyme-catalysed reaction may be coloured or chemiluminescent (i.e. emitting light).

Summary of Section 1.7

- Gel filtration is a chromatographic technique that separates proteins according to size. It can be used as a preparative technique (to purify proteins) or, on a smaller scale, as an analytical technique.
- The techniques of SDS–PAGE and immunoblotting are widely used to analyse the protein composition of mixtures such as cell homogenates, to determine the approximate M_r of proteins and their subunit composition, and to identify proteins.

1.8 Enzymes

Of all the different kinds of proteins in living organisms, the vast majority are enzymes. In the absence of a catalyst, most chemical reactions occur slowly; though, generally speaking, the rate of reaction can be increased by altering physical conditions – for example, by increasing the temperature or pressure or, in some cases, by changing the pH. Without enzymes, most of the biochemical reactions that occur in living organisms would not occur at any detectable rate and it would not be possible to sustain life. Enzymes are biological catalysts, accelerating the rate of reactions to between 10^9 and 10^{14} times the rate of the uncatalysed reaction. By accelerating reactions, enzymes permit biochemical processes to occur in very moderate physical conditions, without requiring extremes of temperature, pressure or pH.

A given cell may be capable of synthesising several thousand enzymes, each one catalysing a different reaction. The range of enzymes is truly immense, but their activities display many common features that are examined in this section.

1.8.1 The key characteristics of enzymes

The chemical upon which an enzyme acts is known as the **substrate**. The substrate binds to a binding site on the enzyme and is chemically transformed to form a product or products. The enzyme-catalysed reaction can thus be represented as follows:

$$\mathrm{E + S \rightleftharpoons ES \rightleftharpoons E + P}$$

where E is the enzyme and ES is an enzyme–substrate complex, formed by binding of the substrate to the enzyme. This is illustrated in Figure 1.16.

■ Is the enzyme altered by the conversion of S into P?

□ No, the enzyme is unchanged.

In fact, an important feature of enzyme-catalysed reactions is that the enzyme is unaltered by the reaction and is freed to catalyse the conversion of more molecules of S into molecules of P.

Looking again at the simple equation for the enzyme-catalysed conversion of substrate into product, it is clear that the first event that must occur (prior to

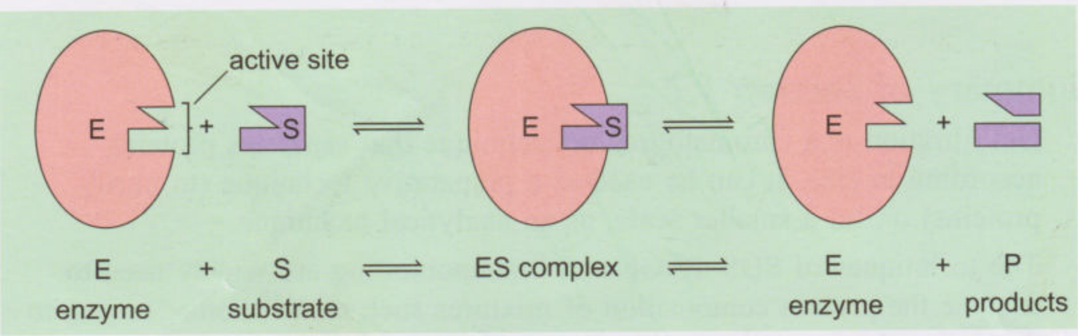


Figure 1.16 Schematic representation of the relationship between an enzyme, its substrate, and the reaction catalysed by the enzyme.

the actual chemical reaction) is the binding of the substrate to the enzyme. The substrate binding site on the enzyme is known as the **active site**, since it is also the site at which the chemical reaction occurs. Enzyme function depends on the exact conformation (shape and three-dimensional structure) and the chemistry of the active site. Gene mutations that alter critical amino acid residues at the active site have severe effects on enzyme activity. Indeed, much of what is known about the mechanisms of specific enzymes has been derived from studies in which alterations in the structure of the active site have been engineered for experimental purposes (see Section 1.9).

The so-called **lock and key hypothesis** proposed by Emil Fischer in 1894 is still a helpful way to describe the enzyme–substrate interaction. In this model, the substrate can be envisaged as a unique key which fits precisely in the enzyme active site (i.e. the lock). It is important, however, to realise that substrate binding is not just a matter of two complementary shapes fitting together like jigsaw pieces; but, because chemical interactions must be formed between the substrate and the enzyme, the two molecules must be chemically complementary, in particular with respect to charged groups.

From Figure 1.16 and the equation above, it should be clear that the steps in the reaction catalysed by an enzyme are generally reversible (indicated by the two-way arrows in the equation). Enzymes can bind their substrate to form an ES complex, which may then immediately dissociate without leading to product formation; alternatively, successful catalysis results in breakdown of the ES complex into enzyme and product(s). In theory, this step is also reversible, but often products do not bind as readily to the enzyme as the substrate, so in practice this stage of the reaction may not go in reverse.

Another important feature of enzymes is their substrate specificity. Some enzymes are highly specific with regard to which substrates they can bind and act on, others less so. For example, hexokinase, which catalyses the first reaction of glucose breakdown (glycolysis, described in Chapter 3) in muscle and other tissues, uses only glucose as its substrate. In contrast, the digestive protease trypsin is less substrate-specific and hydrolyses any peptide bond between the basic amino acids lysine or arginine and the next amino acid on the C-terminal side (with the exception of proline). The very broad substrate specificity of trypsin is related to its function of digestion of dietary protein, which may comprise a very heterogeneous collection of molecules.

Enzymes are also quite specific with respect to the reaction or type of reaction they catalyse. A particular molecule may be the substrate of more than one enzyme, but each of these enzymes catalyses the production of a different product(s). For example, pyruvate is a substrate for lactate dehydrogenase, which catalyses its conversion into lactate, and also for the pyruvate dehydrogenase complex, which converts it into acetyl CoA and carbon dioxide.

For all chemical reactions, regardless of whether they are catalysed or not, an energy barrier must be overcome in order to convert the substrate into products. This barrier represents the energy required for formation of an unstable intermediate known as the transition state. Enzymes work by offering an alternative reaction pathway in which less energy is required to achieve the transition state, thus effectively lowering the energy barrier presented by the reaction and allowing it to proceed more quickly (Figure 1.17); the lower the barrier, the faster the reaction. The degree to which a particular enzyme speeds up a reaction is referred to as its catalytic power.

It is important to realise that although enzymes can speed up the rate at which reactions occur, whether or not a reaction occurs at all depends on whether it is energetically favourable. The meaning of the term 'energetically favourable' will be discussed in Chapter 3.

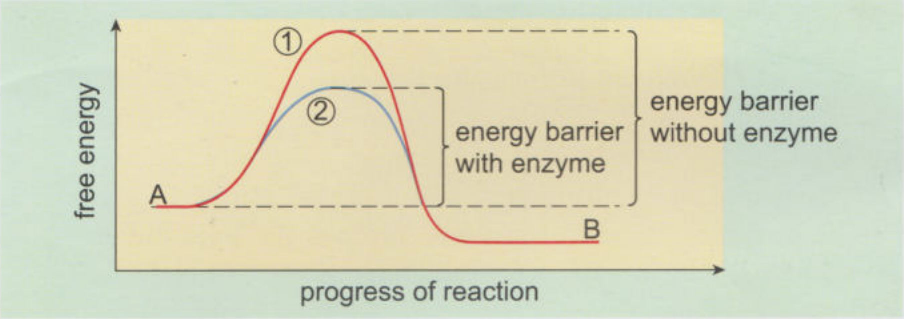


Figure 1.17 The change in energy (known as free energy) during the conversion of A into B: curve 1, without enzyme; curve 2, for the enzyme-catalysed reaction. The peak of each curve denotes the energy of the unstable intervening stage (transition state).

The rate at which enzyme-catalysed reactions actually occur in a cell depends on a variety of factors and, as you will see, conditions such as temperature, pH, or the presence of cofactors or inhibitors can all affect enzyme activity. Before considering these factors in more detail, this section will first focus on the relationship between the rate of an enzyme-catalysed reaction and the concentration of the substrate consumed in the reaction.

1.8.2 Michaelis–Menten enzyme kinetics

The study of the rate at which enzyme-catalysed reactions occur is known as enzyme kinetics (from the Greek *kinetikos*, meaning 'moving'). This is a very complex subject and for the present purposes the focus here is on the relationship between the substrate concentration and the initial rate of a reaction, which is described by **Michaelis–Menten kinetics** and which holds true for most enzymes. It is important to note that over time, the rate of an enzyme-catalysed reaction will decline as the substrate is consumed or the accumulation of product inhibits the conversion of substrate into product (remember that enzyme reactions are often reversible; see Section 1.8.1). However, because it is only concerned with *initial* rates of reaction, Michaelis–Menten kinetics assume that the reduction in the concentration of substrate and the accumulation of product do not significantly affect the rate of reaction. Box 1.6 describes how the rate of enzyme reactions can be measured experimentally using enzyme assays.

Box 1.6 Enzyme assays

Rather than measure the amount or concentration of an enzyme in a preparation, it is generally more appropriate to measure its activity; in other words, the rate of the reaction catalysed by the enzyme. This is known as an enzyme assay, where assay here means 'measurement'.

■ Consider Figure 1.16. What could be measured to assess the activity of a given enzyme?

□ As the enzyme reaction proceeds, substrate is converted into product, so substrate is consumed and product appears. Activity could therefore be assessed by measuring either the rate of removal of substrate or the rate of formation of product.

Quite often it is most convenient to measure the appearance of product as the enzyme reaction proceeds. Products that have particular chemical properties are most readily measured. For example, a coloured product can be quickly measured in a colorimeter, and a gas measured in a gas analyser. In some enzyme-catalysed reactions, protons (H^+) are either consumed or produced, and in such cases changes in pH can be measured. Yet other enzymes interconvert oxidised and reduced forms of coenzymes, which can be distinguished using appropriate techniques.

However a given enzyme's activity is assayed, it is usually expressed as specific activity, which by convention is expressed relative to the amount of protein (in mg, which can be measured chemically) contained in the preparation. **Specific activity** of an enzyme preparation is therefore defined as micromoles of substrate converted into product per minute per mg protein (i.e. $\mu\text{mol min}^{-1}\text{ mg}^{-1}$).

Note that the expression 'micromoles of substrate converted' has a predictable relationship to 'micromoles of product formed'. Thus if one molecule of product is generated for every molecule of substrate used, the rate of conversion of substrate will be the same as the rate of formation of product.

The relationship between the initial rate of an enzyme-catalysed reaction (v) and the substrate concentration ([S]) at the start is represented in graphical form in Figure 1.18. Note that the shape of this graph is a curve or hyperbola.

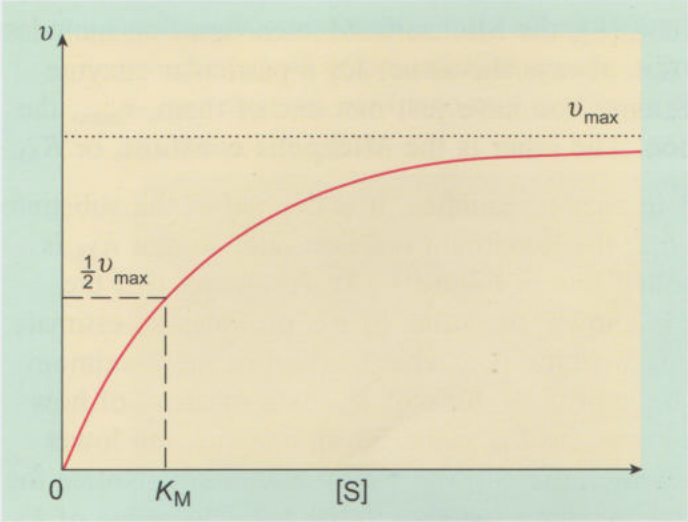


Figure 1.18 Plot of initial reaction rate v against initial substrate concentration [S] for an enzyme-catalysed reaction that follows Michaelis–Menten kinetics.

■ Consider the plot of v versus [S] in Figure 1.18. Describe the relationship between these two variables.

□ At first, v increases steeply with increasing [S], but starts to level off (plateau) at the higher substrate concentrations.

Square brackets are used to denote 'concentration of'. Thus [S] means the concentration of substrate S.

This relationship between v and [S] can be explained as follows. At low values of [S] – that is, when there are many more enzyme molecules than substrate molecules (referred to as an excess of enzyme) – the reaction rate increases as [S] increases, hence the curve rises steeply close to the origin. As [S] increases further, an increasing proportion of the enzyme molecules have a substrate molecule bound to them, and the overall reaction rate becomes more dependent on the rate at which the product is formed, liberating the enzyme again to bind to more substrate; hence the steepness of the slope of the curve decreases. At very high values of [S], when all the enzyme molecules are involved in the reaction – that is, when the enzyme is *saturated* – the reaction rate cannot increase any further (without changing the conditions, such as by adding more enzyme), so the curve becomes an almost horizontal line. The dotted line that intersects with the y-axis in Figure 1.18 represents a maximum initial rate of reaction, $\boldsymbol{v}_{\mathbf{max}}$ (spoken as 'vee max'). Notice that as [S] increases towards infinity, v approaches, but never actually reaches, the dotted line representing $v_{\max}$.

In 1913, two German enzymologists, Leonor Michaelis and Maud Menten, established that the relationship between v and [S] could be described by the mathematical equation:

$$v = \frac{v_{\max}[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]}$$

where v is the initial reaction rate and [S] is the initial substrate concentration.

In addition to the variables v and [S], the Michaelis–Menten equation includes two terms that are a constant (i.e. always the same) for a particular enzyme under a particular set of conditions. You have just met one of them, $v_{\max}$, the maximum initial rate of reaction. The other is the **Michaelis constant**, or K_M.

K_M is a very useful parameter in enzyme kinetics. It is defined as the substrate concentration ([S]) that gives *half* the maximum reaction rate, so that K_M is the value of [S] at ½ $v_{\max}$ (dashed line in Figure 1.18). Assuming that the concentration of the substrate is known, the value of K_M provides an estimate of how effectively an enzyme is working (i.e. what fraction of the maximum rate it is operating at). It can be helpful to think of K_M as a measure of how 'efficient' an enzyme is – the lower the K_M value for an enzyme, the lower the substrate concentrations at which the enzyme works effectively. Notice the large range of K_M values for the enzymes listed in Table 1.2. The value of K_M for an enzyme can often tell you about the physiological concentrations of substrates: low values reflect low substrate concentrations *in vivo* (requiring a very efficient enzyme), and vice versa.

■ Pyruvate carboxylase and lactate dehydrogenase (LDH) both use the same substrate (pyruvate). For a given concentration of pyruvate, which of these enzymes would operate most effectively?

□ LDH has a lower K_M than pyruvate carboxylase and therefore at a given concentration of pyruvate will operate more effectively.

Table 1.2 K_M values for selected enzymes (do not try to remember these; they are just used as examples).

Enzyme	**Substrate**	K_M/µmol l^{-1}
catalase	hydrogen peroxide	1 100 000
carbonic anhydrase	carbon dioxide	12 000
β-galactosidase	lactose	4000
pyruvate carboxylase	pyruvate	400
lactate dehydrogenase (LDH)	pyruvate	59
fumarase	fumarate	5

For some enzymes, K_M is a measure of the 'affinity' of the enzyme for its substrate, i.e. how tightly the substrate binds to the enzyme; the lower the value of K_M, the greater the enzyme affinity. An enzyme with a high affinity for its substrate will efficiently bind its substrate and catalyse the reaction even when substrate concentrations are low.

The numerical value of K_M depends on the reaction conditions. Thus, quantitative analysis of changes in its apparent value caused by pH, temperature, inhibitors, etc., provides valuable insights into enzyme mechanisms. For example, comparison of K_M values also provides a means of comparing the properties of enzymes prepared from different sources.

1.8.3 Factors affecting enzyme activity

Many factors affect the rate of enzyme-catalysed reactions. One of the most important of these, namely substrate concentration, has already been considered. Three others are now considered: temperature, pH and cofactors.

Effects of temperature

Most enzymes function best within a very narrow range of temperature, with activity falling away dramatically either side of the optimum value (Figure 1.19). With increasing temperature, the reaction rate initially increases as molecules of the substrate and the enzyme have more energy and hence move around more, and they therefore have an increased chance of colliding with each other. Another way of describing this effect is that the increase in temperature, which puts more energy into the system, allows more molecules of substrate to overcome the energy barrier. However, above a certain temperature the active, or native, conformation of the enzyme protein is no longer stable. The various non-covalent interactions that hold the enzyme in its native conformation are disrupted, resulting in a huge and usually irreversible drop in activity. In this state, the enzyme is said to be **denatured**.

- ■ What is the optimum temperature of the enzyme activity shown in Figure 1.19?
- □ The enzyme plot shows that its temperature optimum is about 47 °C.

Many mammalian enzymes have temperature optima at 37 °C. In contrast, some prokaryotes that have adapted for life at extreme temperatures have enzymes with optima much lower or higher than those of mammalian enzymes. For instance, some of their enzymes can function optimally at 4 °C, and hence could be highly active in your fridge! Others have temperature optima at 80 °C or even higher, and could function in your hot water tank. You will learn more about organisms that thrive in such extreme temperature conditions in Chapter 2 of Book 3.

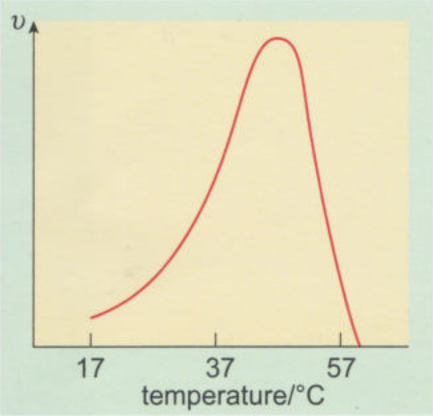


Figure 1.19 Effect of temperature on enzyme activity. The general shape of this curve is characteristic of most enzymes, although peak activity may be very different.

Effects of pH

The effects of pH on enzyme activity are more subtle than that of temperature. You know already that the side chains of many amino acid residues are charged. A pH higher or lower than the optimum value alters the degree of ionisation (dissociation) of ionisable amino acid side chains on the surface of the molecule (and also of those in the substrate). For example, if a particular aspartate side chain is mostly ionised at the enzyme's pH optimum, it will convert into the uncharged form if the pH decreases (i.e. as hydrogen ion concentration increases):

$$-\text{COO}^- + \text{H}^+ \rightarrow -\text{COOH}$$

A change in the pH of the surrounding solution influences all the ionisable groups on the surface of the enzyme, including those that make up the active site and those elsewhere in the molecule that are essential for maintaining the structure of the active site. The combined effect usually reduces enzyme

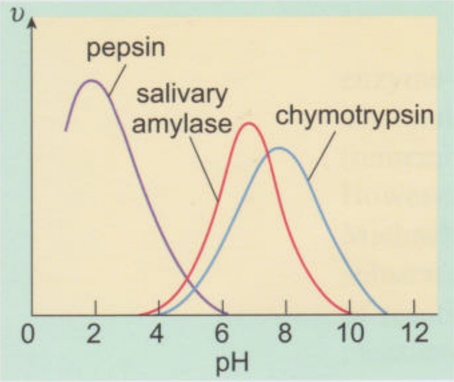


Figure 1.20 Effect of pH on enzyme activity for three mammalian digestive enzymes: pepsin, salivary amylase and chymotrypsin.

activity. Figure 1.20 shows the effects of pH on the activity of three mammalian digestive enzymes.

- ■ From Figure 1.20, estimate the pH optima for the three enzymes.
- □ Estimates for pH optima are: pepsin, pH 2.0; salivary amylase, pH 7.0; and chymotrypsin, pH 8.0.

As a general principle, pH optima for enzymes are related to the environment in which they function. Thus the pH optimum of pepsin is such that the enzyme works efficiently in the highly acidic conditions of the vertebrate stomach where it is secreted. As you will learn in Chapter 2 of Book 3, some prokaryotes thrive at very extreme pH because their enzymes function optimally under these conditions.

Cofactors

As mentioned earlier (Section 1.5.1 and in Activity 1.1), many proteins have associated with them non-protein molecules or ions that are essential to their function. In some cases, these cofactors are permanently associated with the protein (in which case they are often referred to as *prosthetic groups*). Haem, which associates with each of the globin subunits in haemoglobin, is an example of a cofactor that is in fact a prosthetic group.

Many enzymes require appropriate concentrations of specific cofactors for their activity. The activity of up to a third of all enzymes depends on the presence of a particular bound metal ion such as Cu^{2+}, Fe^{2+}, Na^{+} or Mg^{2+}.

Coenzymes are cofactors that include NAD, ATP (Book 1, Section 1.2.1) and ADP (a molecule identical to ATP but with one less phosphate). The coenzymes are a diverse group of organic molecules required for the function of many enzymes. Most coenzymes, like substrates, bind reversibly at a specific place in the active site of the enzyme, and are actively involved in catalysis by accepting or donating specific chemical groups.

1.8.4 Enzyme inhibition

Because the specific interaction of the enzyme with its substrate(s) is critical to its function, anything that interferes with this interaction will effectively inhibit the catalytic activity of the enzyme. The effects of two different kinds of enzyme inhibitor, competitive and non-competitive, on the rate of the enzyme-catalysed reaction are illustrated in Figure 1.21. **Competitive inhibitors** are molecules that can bind to the active site of an enzyme and block access of the substrate, i.e. they compete with the substrate for binding to the active site (Figure 1.22). Other inhibitors, known as **non-competitive inhibitors**, do not prevent the substrate from binding but compromise the ability of the enzyme to catalyse the reaction. On binding to a site on the enzyme that is not the active site, non-competitive inhibitors alter the conformation of the active site in such a way that the enzyme cannot catalyse the reaction as efficiently as it would do otherwise.

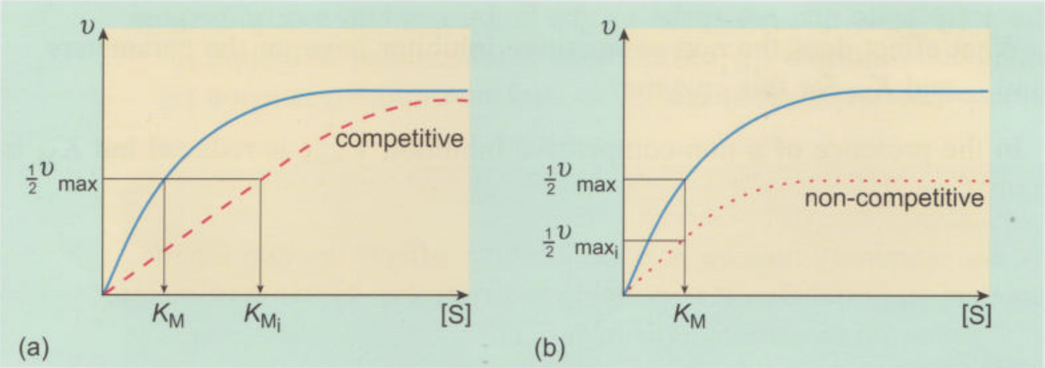


Figure 1.21 (a) Plot of the initial rate of an enzyme-catalysed reaction (v) against concentration of substrate ([S]) in the presence of a competitive enzyme inhibitor (dashed line). The plot for the same enzyme in the absence of inhibitor is also shown (solid line). (b) Similar plots in the presence of a non-competitive enzyme inhibitor (dotted line) and in the absence of inhibitor (solid line).

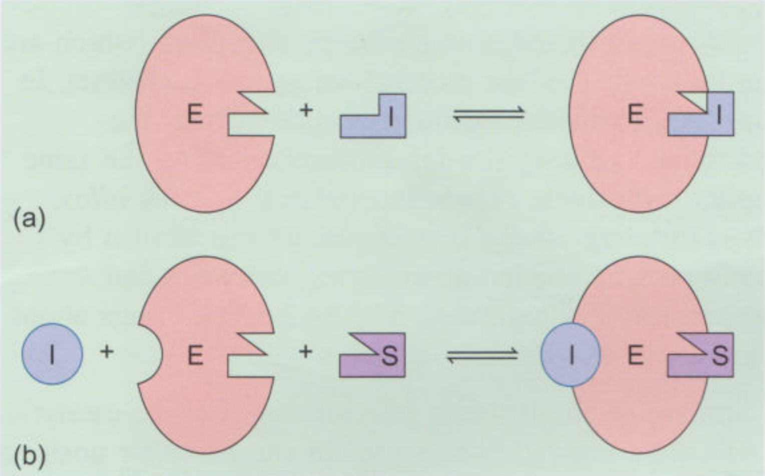


Figure 1.22 Schematic illustration of the mechanism of competitive and non-competitive inhibition (I = inhibitor). (a) A competitive inhibitor competes with the substrate for binding to the active site. (b) A non-competitive inhibitor binds to a different site on the enzyme than does the substrate; in doing so, it forms an inactive complex which, even if the substrate binds, cannot catalyse the reaction.

■ Consider Figure 1.21. For each of the two different types of enzyme inhibitor, describe how it affects the relationship between v and [S].

□ The competitive inhibitor causes the curve to shift to the right, i.e. higher concentrations of substrate are required to obtain a rate equivalent to that in the uninhibited reaction. The non-competitive inhibitor reduces the rate of reaction regardless of how much substrate is present, and the maximal rate (i.e. the rate at which the curve plateaus) is thus reduced.

■ What effect does the competitive inhibitor have on the parameters $v_{\max}$ and K_M for this enzyme?

□ In the presence of a competitive inhibitor, $v_{\max}$ is unaffected but K_M is increased.

- ■ What effect does the non-competitive inhibitor have on the parameters $v_{\max}$ and K_M for this enzyme?

- □ In the presence of a non-competitive inhibitor, $v_{\max}$ is reduced but K_M is unaffected.

Thus, by examining how an enzyme inhibitor affects the two kinetic parameters $v_{\max}$ and K_M, it is possible to determine its mode of action.

1.8.5 Regulation of enzyme activity

There are two principal mechanisms by which the activity of enzymes is regulated – allosteric regulation and reversible covalent modification (in particular phosphorylation) – which are described below. Note, however, that these regulatory mechanisms apply to proteins in general, not just to enzymes.

Allosteric regulation

A protein may bind more than one ligand, though the binding sites (which are often clefts or pockets on the surface of the protein) are generally distinct. In many proteins, the binding of a particular ligand at one site affects the conformation of a second remote binding site for another ligand on the same protein. This is an example of **allosteric regulation** (from the Greek *allos*, meaning 'other' and *steros*, meaning 'shape'), an important mechanism by which the function of many proteins, including enzymes, receptors and components of signalling pathways, is regulated. The ligand that brings about this regulatory effect is known as an *allosteric effector.*

In the case of enzymes, binding of an allosteric effector can alter the catalytic activity of the enzyme. Allosteric regulation of a protein can be either positive or negative, increasing or decreasing the protein's affinity for its ligand; or, in the case of an allosterically regulated enzyme, its catalytic activity. Allosteric regulation affects enzyme activity by changing the conformation of the enzyme, specifically changing the shape and substrate-binding properties of the active site. An example of an allosteric enzyme is aspartate transcarbamoylase (ATCase) in *E. coli*, which catalyses the first step in the synthesis of a nucleic acid precursor called cytidine triphosphate (CTP). ATCase is a multimeric enzyme comprising six catalytic subunits and six regulatory subunits. CTP, the end product of the pathway, inhibits ATCase by binding to the regulatory subunits and causing a change in their conformation which in turn affects the conformation of the active site on the catalytic subunits.

Reversible covalent modification

As discussed in Section 1.6.1, some proteins undergo permanent covalent modifications – the linking of sugars or lipids to proteins to form, respectively, glycoproteins and lipid-linked proteins. However, some covalent changes in proteins are only temporary, and **reversible covalent modification** represents an important means of regulation of protein function.

■ Can you recall an example of covalent modification of a protein from Book 1?

□ An example is the acetylation of histones, the proteins that associate with DNA in chromatin (Book 1, Section 6.4.4). By affecting the packaging of the DNA and ultimately its accessibility to the transcriptional machinery, this covalent modification is an important means by which transcription of particular genes is regulated.

Note that methylation is another form of reversible covalent modification that is observed in histones in eukaryotic cells. As with acetylation, methylation of histones (at their N-terminal tails) can affect the structure of chromatin and ultimately gene expression.

Phosphorylation

The addition of a phosphate group, or **phosphorylation**, is a particularly important reversible covalent modification of proteins. Phosphorylation of the –OH group in the side chains of serine, threonine and tyrosine is catalysed by enzymes known as protein kinases. The reverse reaction, dephosphorylation, removes the phosphate group, and is catalysed by a protein phosphatase (Figure 1.23). Eukaryotic cells have hundreds of enzymes that are regulated by reversible phosphorylation/dephosphorylation of specific amino acids (and many different protein kinases and protein phosphatases).

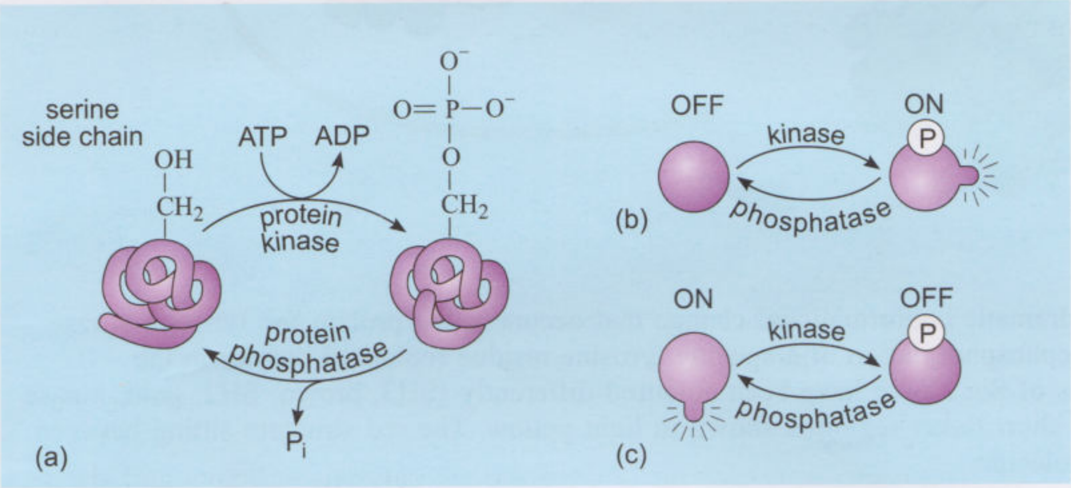


Figure 1.23 Schematic picture of protein phosphorylation. (a) The general reaction entails transfer of a phosphate group from ATP to an amino acid side chain of a target protein by a protein kinase. Removal of the phosphate group is catalysed by a second enzyme, a protein phosphatase. In this example, the phosphate is added to a serine side chain. The phosphorylation of a protein by a protein kinase can either increase (b) or decrease (c) the protein's activity, depending on the site of phosphorylation and the structure of the protein.

How can addition of a small phosphate group bring about such dramatic changes in the activity of a protein? The key is the chemical nature of the phosphate group, which is strongly negatively charged. Phosphorylation therefore alters the balance of non-covalent interactions that determine the higher-order structure of the protein. The resulting conformational change may cause activation or inactivation of the protein's function (e.g. enzyme activity), or association or dissociation of subunits. A good illustration of this effect is the dramatic switch between the 'open' active conformation of Src tyrosine kinase and the 'closed' inactive conformation which this enzyme adopts when a specific tyrosine residue in the C-terminal tail is phosphorylated (Figure 1.24). When phosphorylated at this location, the tail interacts with the SH2 domain and the protein closes up, blocking the active site and the part of the SH3 domain that binds substrates and brings them close to the active site. (The domain structure of Src tyrosine kinase was explored in Activity 1.1.)

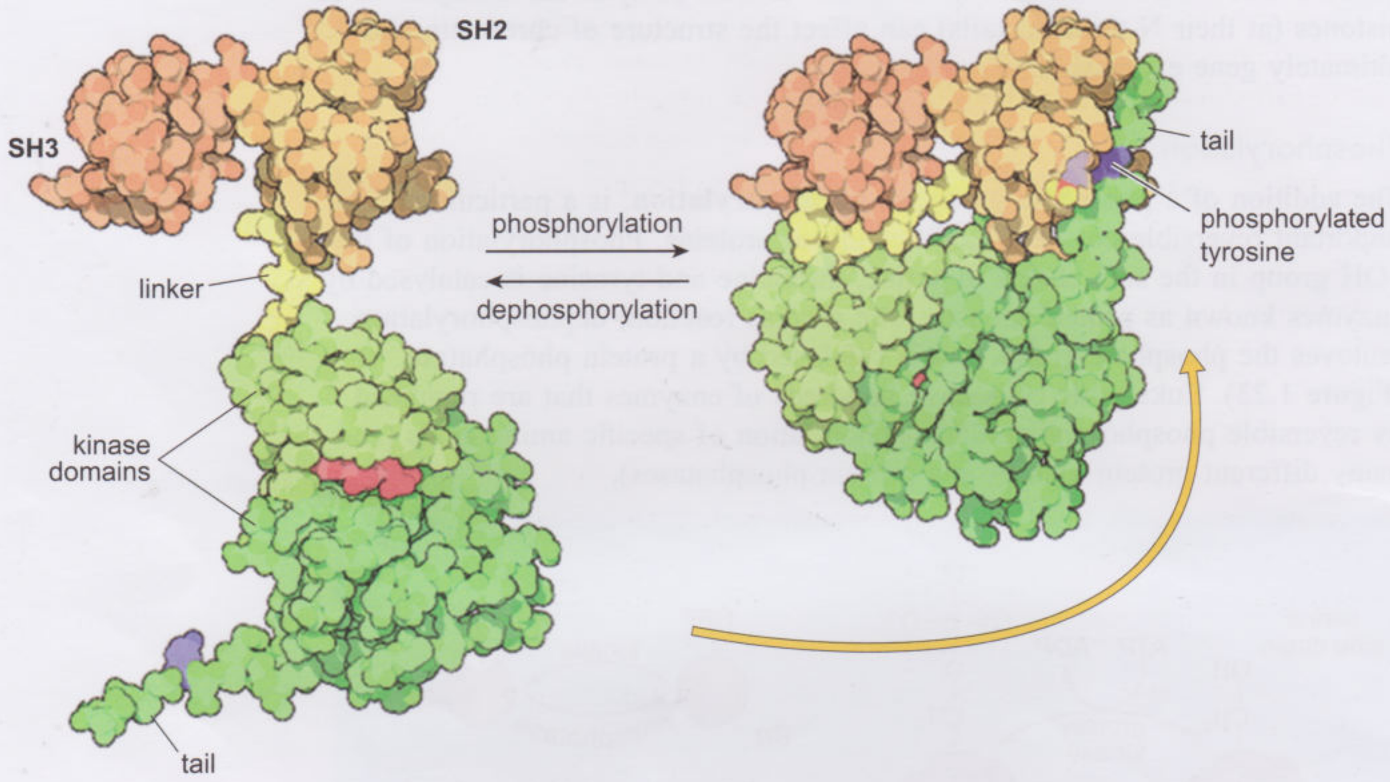


Figure 1.24 Representation of the dramatic conformational change that occurs in the protein Src tyrosine kinase when it undergoes phosphorylation/dephosphorylation of a specific tyrosine residue (coloured purple) in the C-terminal tail. Note that the domains of Src kinase have been coloured differently (SH3, brown; SH2, gold; kinase domains, two shades of green) and a short linker region is shown in light yellow. The red structure sitting between the two kinase domains is an ATP molecule.

There are other reversible covalent modifications that occur in proteins. In plants, amino acid side chain reactions are also important in the control of enzyme activity. A commonly used reversible covalent modification involves the disulfide bonds that link pairs of cysteine residues. The breaking of a disulfide bond activates certain chloroplast enzymes that are involved in carbohydrate synthesis.

Reversible covalent modification, notably protein phosphorylation, plays a major role in cell functions and you will encounter numerous examples throughout this module. Chapter 3 will consider some aspects of cellular metabolism, the complex network of enzyme-catalysed reactions that occur in cells. In doing so, it will revisit enzyme regulation, which is critical to the control of metabolic processes and hence the survival and function of cells.

Many cells of multicellular organisms, and colonies of unicellular prokaryotes, communicate with one another via signal molecules that transfer information. Processing of this information and responses to it generally involve cycles of phosphorylation/dephosphorylation (Chapter 4). Cell movement, again a feature of many prokaryotic and eukaryotic cells, is also dependent on protein phosphorylation (Chapter 5).

Summary of Section 1.8

- The vast majority of proteins in living organisms are enzymes, biological catalysts.
- The substrate binds at the active site of an enzyme, forming the enzyme–substrate complex. Catalysis occurs and products are released. Conformations of the active site and substrate are complementary; hence their interaction is described as a 'lock and key' mechanism.
- Enzymes usually exhibit substrate specificity, and bind only a single or limited range of substrates.
- The study of the rates of enzyme-catalysed reactions is known as enzyme kinetics. The relationship between the initial rate of an enzyme-catalysed reaction (v) and the initial concentration of substrate ([S]) is given by the Michaelis–Menten equation: $v = v_{\max}[S]/(K_M + [S])$.
- This relationship includes two terms that are constant for a particular enzyme under a particular set of conditions: $v_{\max}$ is the maximum rate of reaction and K_M is the Michaelis constant, which is defined as the value of [S] at which the rate is ½ $v_{\max}$.
- The lower the K_M for an enzyme, the lower the substrate concentrations at which it works effectively (i.e. the more efficient the enzyme).
- Enzyme activity is affected by various factors, including temperature, pH and cofactors.
- Enzymes can be inhibited by a range of substances. Reversible inhibitors are either competitive or non-competitive. Competitive inhibitors bind at the active site and increase the value of K_M; non-competitive ones bind at sites other than active sites and lower the value of $v_{\max}$.
- Enzymes are subject to regulation by two principal mechanisms: allosteric regulation or reversible covalent modification (in particular, protein phosphorylation).
- Reversible covalent modification of proteins – for example, by addition of phosphate, acetyl or methyl groups – is an important mechanism in regulation of protein function. In particular, cycles of phosphorylation and dephosphorylation are important in regulating the activity of many enzymes and components of intracellular signalling pathways.

1.9 The structure–function relationship in proteins

At the start of this chapter, it was asserted that the key to how a protein performs a particular function lies in its structure, which is critical to protein–

ligand interactions. Enzymes, discussed in the previous section, are a good illustration of this principle. Particularly critical is the structure of the active site of an enzyme, where the substrate binds and catalysis occurs. The structure of a site at which an allosteric effector binds would also be critical to an enzyme's function.

Of course, enzymes are just one type of protein and the ability to bind and interact with specific ligands is equally important in other types of protein. Common ligands that bind to proteins include cofactors (Sections 1.5.1 and 1.8.3) and small molecules such as water (H_2O), oxygen (O_2) and glucose. Proteins also bind to other macromolecules – other proteins, lipids, polysaccharides and nucleic acids. Such interactions can be transient or fairly stable. Stable interactions between proteins and other macromolecules are evident in particular cellular structures such as: ribosomes, which are composed of several specific proteins and RNA molecules; biological membranes, which contain lipids and proteins; and eukaryotic chromosomes, which are complexes of DNA and specific proteins.

All protein–ligand interactions are driven by the formation of non-covalent interactions – hydrogen bonds, ionic interactions and hydrophobic interactions – the same kinds of non-covalent interactions that stabilise tertiary structure (Figure 1.6). Thus protein–ligand interactions depend on the chemical and physical compatibility of the protein and its ligand. For some proteins, altering even a single critical amino acid residue can have a significant effect on the activity of the protein. This point is illustrated by naturally occurring mutations that can result in defective proteins.

■ In which parts of (i) an enzyme, and (ii) a cell surface receptor, might alteration of an amino acid affect the protein's function?

□ (i) In an enzyme, the amino acid residues that are important for binding the substrate at the active site and catalysing its conversion into product would be particularly important, though there may be other residues that are critical for regulation of the enzyme or its association with other proteins or structures. (ii) In a cell surface receptor, the residues whose alteration might affect function are those that participate in the interaction between the receptor and its extracellular ligand, or between cytosolic regions of the receptor and any intracellular signalling molecules.

In addition to these examples, a mutation that changed an amino acid residue that is required to stabilise the folded structure of a protein, or to target it to an appropriate location in the cell, would be expected to affect its activity.

Site-directed mutagenesis (SDM) is a very powerful technique in the study of protein function, which involves the use of recombinant DNA techniques to selectively replace the residue of interest with a different amino acid with critically different properties. In this way, the experimenter can assess the importance of particular amino acid side chains in a protein. SDM is most commonly used in the study of enzymes; however, it is also very useful in identifying key residues in protein–protein interactions. Provided that the sequence of the gene encoding the protein of interest is available and there is a suitable system for expressing the gene, it is possible, using recombinant

DNA technology (discussed in Chapter 4 of Book 3), to produce a mutant protein in which a specific amino acid has been replaced with a different amino acid. The mutant gene can be expressed in large quantities in cells and the resulting mutant protein can then be analysed with regard to its structure and function (e.g. catalytic activity) and comparisons made with the normal (wild type) protein.

The selection of an amino acid for mutation is often based on detailed knowledge of the protein's three-dimensional structure. For example, from the X-ray structure of an enzyme bound to its substrate molecule (see Box 1.3), amino acids likely to be involved in binding the substrate or catalysing the reaction might be identified on the basis of their proximity to the substrate and their chemical properties. These residues are interesting targets for mutagenesis. Similarly, identification of highly conserved residues in comparisons of primary amino acid sequences of related proteins can indicate important roles for these in the protein and can form the basis for an informed choice of target residues for mutagenesis.

The choice of the replacement residue generally depends on the supposed role of the residue in question. For example, to test the importance of a particular residue in an enzyme mechanism, it could be replaced with one of similar overall size but differing in its chemical characteristics.

■ Why do you think it would be advisable to use a residue of similar size for such a substitution?

□ Substitution of an amino acid residue with a larger residue could result in steric interference (Box 1.2), which might affect the overall structure and confound observation of the effect of changes to the chemistry of the residue. It is preferable to use a residue that is smaller than the wild type residue as these tend not to disrupt the overall structure of the protein.

■ What do you think would be an appropriate residue with which to replace a glutamate residue to test whether it is involved in the catalytic mechanism of an enzyme?

□ A glutamate residue could be replaced by glutamine, which is similar in size and shape but lacks the negative charge (Table 1.1).

To illustrate the application of SDM in the study of protein function, the enzyme lysozyme will be considered. This enzyme, which is found in many secretions of vertebrates (including tears, saliva and gastric secretions) and in egg white, catalyses the hydrolysis of bonds between sugar residues in particular kinds of polysaccharide. Owing to its action on the peptidoglycan of the bacterial cell wall (Book 1, Section 3.3), lysozyme can act as a bactericide. The mechanism of the catalytic function of lysozyme has been determined through experimental techniques that have included SDM. Indeed the substitution of one particular glutamate residue with a glutamine (the substitution suggested above) identified that glutamate residue as being essential for the enzyme's activity. Interestingly, the mutant enzyme still bound the substrate molecule but it failed to catalyse the reaction, illustrating that binding of the ligand (a substrate molecule in this case) is distinct from catalysis.

Summary of Section 1.9

- The key to the relationship between protein structure and function is the requirement of all proteins to bind to other molecules, referred to as ligands. These ligands may be ions, small organic and inorganic molecules, or macromolecules (proteins, lipids, polysaccharides, nucleic acids).
- Protein–ligand interactions are specific and are driven by formation of non-covalent interactions.
- The relationship between protein structure and function is demonstrated by site-directed mutagenesis (SDM) experiments in which single critical amino acid residues are altered in a protein, with disastrous consequences for its activity.

1.10 Protein families and structural evolution

In parallel with the evolution of living organisms, the proteins within organisms have themselves evolved over time to generate the huge variety that exists today, driving evolution at the organismal level.

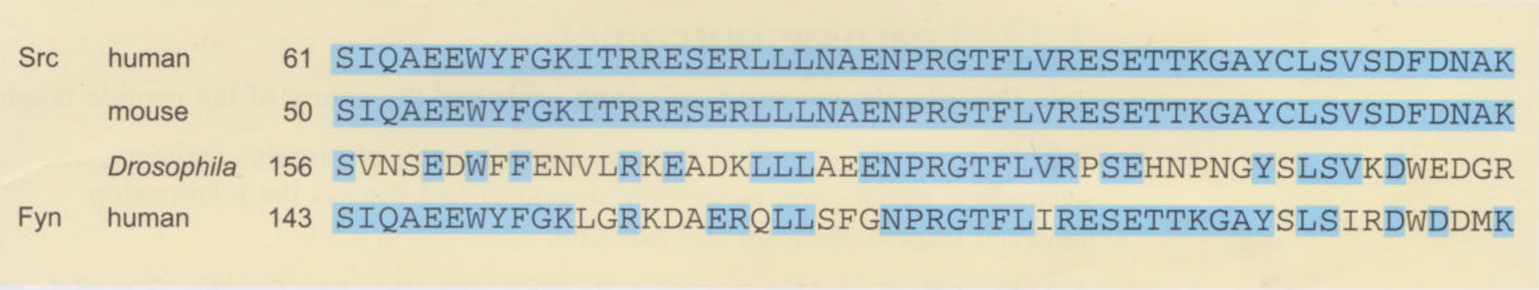
The morphological diversity of extant organisms is paralleled by diversity in protein sequence. The availability of genomic sequence data from every major taxonomic group of organisms has allowed extensive comparisons to be made between their protein-coding regions. From these comparisons, it has become apparent that there is extensive similarity between the amino acid sequences of many proteins, even between apparently distantly related organisms. In some proteins, this extends across the entire protein; in others, it lies within small regions which are called **conserved domains**. Proteins that share a common ancestor protein are described as **homologous**. (Where the proteins are from different species, they may be more specifically described as *orthologous*.)

Consider two genes, from two species, encoding proteins that have 50% of their amino acid sequence in common.

- ■ How can this be explained in terms of evolution?
- □ The most likely explanation is that the similarities result from the fact that the two species share a common evolutionary past and that the genes encoding the proteins in each arose from a common ancestral gene, present in the last common ancestor of the two species.

It is fascinating to consider that ancestors of most proteins that exist today were already present in the last universal common ancestor (LUCA) 3500 Ma (3.5 billion years) ago. Through the process of natural selection (introduced in Section 1.1 of Book 1), randomly occurring DNA mutations that may alter amino acids within the protein and thereby have a detrimental effect upon the organism are selected against; in other words, they are less likely to be transmitted to the next generation and are ultimately lost from the population. As a consequence of natural selection, differences can accumulate between the two proteins; some might enhance or modify function, some might have no effect, but critical amino acids will often remain unaltered. Over many millions of years of evolution, this process results in proteins in which regions critical for function are highly conserved. Indeed, comparative studies of gene and protein sequences provide some of the strongest support for common ancestry.

The biochemical function of many conserved protein domains has been determined; they may, for example, confer a particular catalytic activity or binding activity. This means that for any particular protein, it is often possible to predict aspects of its biochemical function solely by examining the conserved domains that it contains. Figure 1.25 shows the amino acid sequence of a conserved domain that is found in a number of enzymes that belong to what is known as the Src family of tyrosine kinases. (You have already encountered Src kinase, one of the members of this family of proteins, and learnt about its domain structure in Activity 1.1; and regulation of the activity of this enzyme by phosphorylation was described in Section 1.8.5.) Equivalent parts of the amino acid sequences of the SH2 domain in human, mouse and *Drosophila* Src kinase and human Fyn kinase (another member of the Src family) are shown aligned, and the residues that are identical to those in human Src kinase are highlighted. Sequences in which 30% or more of the residues are identical are generally said to demonstrate homology.



Src	human	61	SIQAEEWYFGKITRRESERLLLNAENPRGTFLVRESETTKGAYCLSVSDFDNAK
	mouse	50	SIQAEEWYFGKITRRESERLLLNAENPRGTFLVRESETTKGAYCLSVSDFDNAK
	Drosophila	156	SVNSEDWFFENVLRKEADKLLLAEENPRGTFLVRPSEHNPNGYSLSVKDWEDGR
Fyn	human	143	SIQAEEWYFGKLGRKDAERQLLSFGNPRGTFLIRESETTKGAYSLSIRDWDDMK

Figure 1.25 Partial amino acid sequence alignment of the SH2 domain from two members of the Src family of tyrosine kinases: Src (sequence data are shown for human, mouse and *Drosophila*) and Fyn (human sequence only). Amino acid residues identical to those in the human Src kinase sequence are highlighted in blue. Note that the single letter code for amino acids is used (see Table 1.1).

Before the advent of modern molecular techniques, the function of a newly identified protein would be investigated through a number of different techniques designed to elucidate its chemical, physical and biochemical properties. For example, its behaviour under various purification protocols, which depend on its chemical and physical properties, would be determined; if pure samples could be obtained, its structure would be examined using techniques such as X-ray crystallography or NMR spectroscopy; and biochemical assays would be performed to test its activity. Determining the amino acid sequence of a protein was at that time a relatively low priority in characterising a newly identified protein. Now, however, it is typically top of the list and is usually deduced from the DNA sequence of the gene that encodes the protein. Identification of similarity between a protein's sequence and that of other 'known' proteins is, in fact, the most powerful means of inferring the function of a protein. Using this sort of information, a rational experimental approach can be developed to test predictions about a protein's likely function.

Summary of Section 1.10

- Knowledge of the amino acid sequence of a protein (generally derived from DNA sequence data) is very useful when trying to determine its function. A high level of similarity between the sequences of two proteins is likely to indicate that they are homologous, i.e. they share a common ancestor protein, and may have similar functions.

1.11 Final word

Having studied this chapter, you should be able to appreciate how polypeptides fold in a consistent manner to make mature functional proteins and how this process depends on the sequence of amino acids in the polypeptide and their chemical and physical properties. The function and regulation of enzymes have been described in detail here. However, as you study the rest of this module, you will encounter many different proteins doing many different jobs in the cell, and some common principles, highlighted in this chapter, apply equally to these proteins.

1.12 Learning outcomes

1.1 Describe the chemistry of amino acids and the nature of the peptide bond.

1.2 Describe the structural features and hierarchy of proteins (primary, secondary, tertiary and quaternary structure) and discuss the relationship between protein structure and function.

1.3 Identify secondary structural elements and other key features in models of protein structures.

1.4 Describe how polypeptides fold to make mature functional proteins.

1.5 Describe the application of, and understand the principles of, techniques for isolating proteins and for the study of protein structure and/or function, including gel filtration, X-ray crystallography, SDS–PAGE, immunoblotting and site-directed mutagenesis.

1.6 Interpret the results from SDS–PAGE and immunoblotting experiments.

1.7 Describe how enzymes work and use them to illustrate the structure–function relationship that exists for proteins in general.

1.8 Understand and describe the relationship between v and [S] given by the Michaelis–Menten equation, and explain the significance of v_{max} and K_{M}.

1.9 Describe how enzyme activity is affected by temperature, pH and enzyme inhibitors, and how it is regulated.

1.10 Discuss protein–ligand interactions, their importance in protein function and their dependence on protein structure.

1.11 Discuss the significance of amino acid sequence homology and structural similarities in proteins in the context of protein evolution.

Chapter 2 Membranes and transport

2.1 Introduction – the importance of membranes

One of the key messages of the previous chapter is that the functioning of all cells depends on the activities of their constituent proteins; that is, the expression products of their genes. In this chapter, you will see that membranes are also fundamental to cell function. The function of many proteins depends on their being part of the structure of a membrane. In particular, membranes, by compartmentalising particular biochemical activities, enable many enzymes to work in sequence and in the required chemical environment; they support the large protein molecules that selectively transport materials into and out of cells, transduce energy, and allow cells to communicate with each other and recognise their neighbours; and in animal cells, membranes act with the cytoskeleton to help maintain and adjust cell shape.

The outermost membrane, known as the cell membrane (sometimes referred to as the plasma membrane) is a feature of all cells. Without the exchange of materials and signals between cells, under the control of the cell membrane, eukaryotic cells would be unable to differentiate and function as part of multicellular organisms. Unlike most prokaryotes, eukaryotic cells have intracellular membranes, which separate the cell contents into different regions; that is, the various membrane-bound compartments and the cytosol.

- ■ What are these membrane-bound compartments collectively known as? Give some examples of these.

- □ They are called organelles (described in Book 1, Chapter 3). Examples include the nucleus, mitochondria, chloroplasts, Golgi apparatus, endoplasmic reticulum, lysosomes.

Membranes are a means of controlling the movement of substances (nutrients, waste products, ions) into and out of cells and between intracellular compartments. This control makes it possible for the cell to regulate the composition of the cytosol and the fluid within individual organelles, and it is dependent on a very specific property of cell membranes: their selective permeability. In other words, some substances can cross membranes easily and others cannot. Compartmentalisation within cells allows cellular functions to be partitioned in specific biochemical environments – for example, by restricting a particular enzyme to a particular membrane-bound compartment and maintaining the appropriate concentrations of ions and small molecules that create the chemical environment required for that enzyme to function correctly. The compartmentalisation achieved by intracellular membranes was an important stage in the evolution of the eukaryote cell since it permitted increased complexity and specialisation.

You will learn more in subsequent chapters about the cellular processes in which membranes play a crucial part; but in this chapter, the emphasis is on the main functions of biological membranes, the membrane features and properties on which these functions depend, and how these properties in turn depend on the biochemical composition of membranes.

2.2 Membrane structure

Biological membranes are made of three major components – lipids, proteins and carbohydrates. The biological function of membranes is directly related to the biochemical and biophysical properties of membrane components. Apart from the relative proportions of lipid, protein and carbohydrate in membranes, the specific molecular composition, i.e. which proteins and lipids are present, can vary considerably. This is particularly true of the protein component, which reflects the specialised function of cells and organelles and is often subject to change in the lifetime of the cell, for example during differentiation.

In electron micrographs of cells, such as those in Book 1, Chapter 3 and in Figure 2.1a below, cell membranes are visible as a three-layered dark–light–dark sandwich-like structure of total thickness around 6–9 nm. This appearance is a consequence of a bilayer arrangement of constituent lipids, principally phospholipids. Phospholipids have a polar (i.e. hydrophilic or 'water-loving') head group and a long non-polar (i.e. hydrophobic or 'water-hating') tail. Molecules such as phospholipids, which have both a hydrophilic and a hydrophobic portion, are described as **amphipathic**.

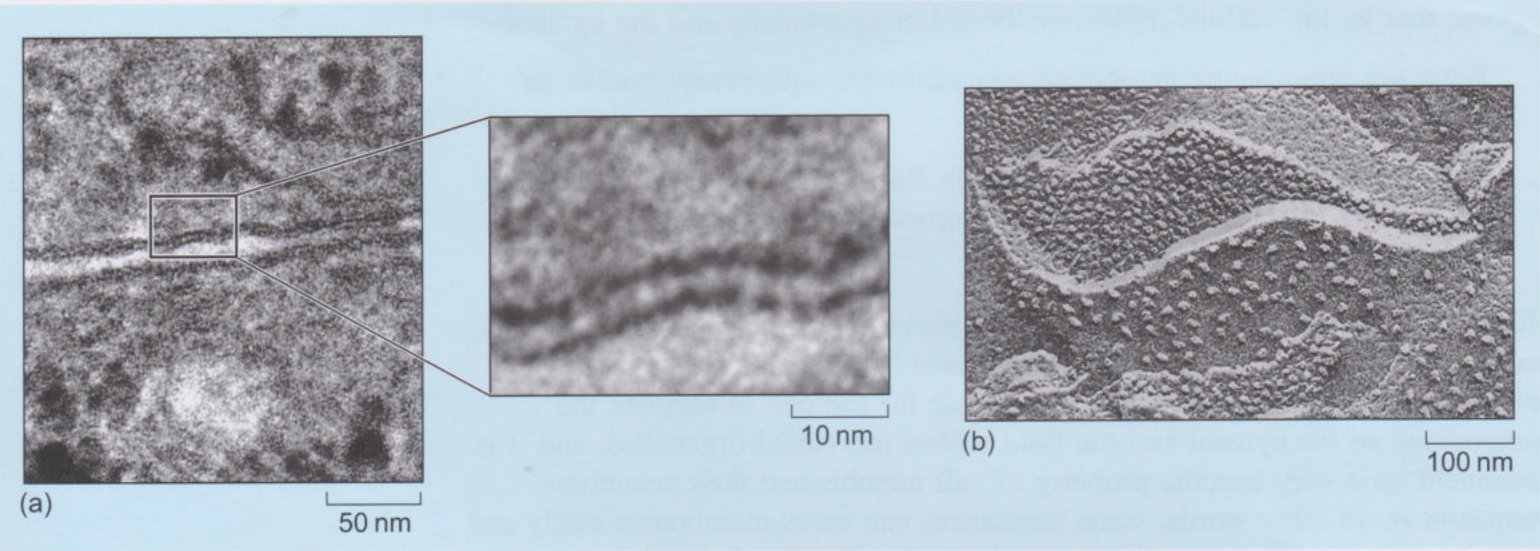


Figure 2.1 (a) A transmission electron micrograph of two closely apposed liver cells, in which the cell membrane can be seen as having a three-layered dark–light–dark sandwich-like structure (shown in the magnified area). The specimen is stained with heavy metals as described in Book 1, Section 3.2.1. (b) A scanning electron micrograph of a membrane (a thylakoid membrane from a chloroplast) that has been prepared using a freeze-fracture method that reveals the 'surface' of the layers in a membrane. In the freeze-fracture technique, rapidly frozen membranes are fractured with a cold knife such that the bilayer splits into two monolayers.

Most individual membrane lipids, including phospholipids, actually have two hydrophobic tails, as depicted in Figure 2.2a. In the membrane, the heads of the phospholipids form the two dark layers observed by electron microscopy (EM) (the 'bread' in the sandwich-like structure), and the hydrophobic tails, which project from the head groups towards the interior of the bilayer, form the 'filling' in the sandwich. Interspersed among the lipids are proteins, which can be seen as bumps in scanning EM images such as Figure 2.1b.

The preparation of artificial membranes (Box 2.1) has facilitated a great deal of research on the structure, properties and functions of lipid bilayers.

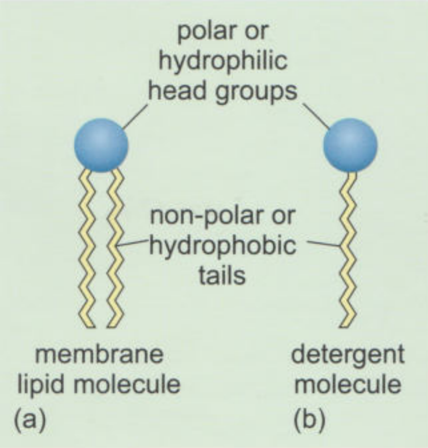

polar or hydrophilic head groups
non-polar or hydrophobic tails
membrane lipid molecule
(a)
detergent molecule
(b)

Figure 2.2 (a) Schematic representation of an amphipathic membrane lipid molecule showing its hydrophilic head group and two hydrophobic tails. (b) Detergents are also amphipathic but have a single hydrophobic tail.

Box 2.1 Liposomes and artificial lipid bilayers

When mixed with water, amphipathic lipid molecules spontaneously form structures in which the hydrophilic head groups have maximum contact with the water, and the hydrophobic tails have minimal contact with water. As with protein folding, which was discussed in the previous chapter, lipid aggregation is driven by the need to achieve maximal stability (i.e. minimal energy) of the amphipathic lipid molecules.

- ■ From what you have learnt about non-covalent interactions (Chapter 1), what interactions do you think are important in the formation of the lipid bilayer?
- □ Hydrophobic interactions form between the non-polar hydrophobic tails; the polar hydrophilic head groups form electrostatic interactions and hydrogen bonds with each other and with surrounding water molecules.

Through the formation of these non-covalent interactions, the two-tailed amphipathic lipids characteristic of biological membranes (Figure 2.2a) will spontaneously aggregate in an aqueous medium to form a bilayer structure in which the hydrophobic tails point inwards and the polar head groups are in contact with each other and the surrounding aqueous medium. Because an open-ended bilayer with exposed hydrophobic areas at its edges would be unstable, the bilayer forms a sphere known as a **liposome** (Figure 2.3). Liposomes are quite stable and can be isolated and manipulated using a variety of standard laboratory techniques. Using electron microscopy, the appearance of sectioned liposomes is a dark–light–dark sandwich structure, like that of similarly prepared biological membranes. Note that the liposome encloses some of the aqueous medium, effectively separating it from the rest of the medium outside the liposome.

Detergents are routinely used by cell biologists to destabilise lipid bilayers and hence disrupt cell membranes and release the cells' contents for study. Detergents, like phospholipids, are amphipathic lipid molecules; however, because they only have one hydrophobic tail (Figure 2.2b), in an aqueous environment they pack together not in a bilayer, but in spherical micelles in which the core is occupied by the hydrophobic tails, and hydrophilic head groups cover the surface. Detergent molecules can effectively insert into a membrane and capture and remove membrane lipids and proteins.

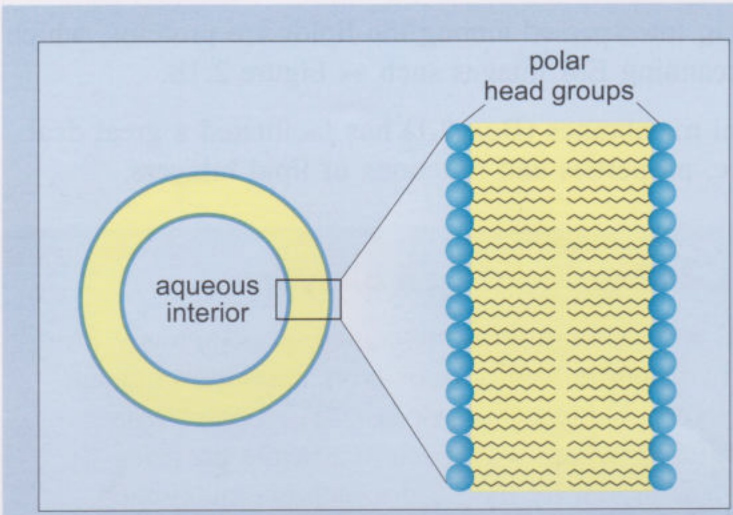


Figure 2.3 In an aqueous medium, amphipathic membrane lipids spontaneously aggregate to form liposomes; one is shown here in cross-section. Liposomes are spherical structures containing the aqueous medium, bounded by a lipid bilayer (shown in enlarged area).

It was not until the 1950s that electron microscopy techniques were developed to allow researchers to actually visualise biological membranes. From biochemical studies, it became clear that there was considerable variety in the composition of different membranes (see Table 2.2 in Section 2.4), an observation that was at odds with the remarkably similar appearance of these membranes when examined by EM. With the development and application of freeze-fracture techniques (see Figure 2.1b), it was clear that the inner faces of the monolayers are studded with globular proteins. In 1972 Jonathan Singer and Gareth Nicolson proposed what is known as the fluid-mosaic model of membrane structure which, with some refinements (discussed in Section 2.6), is still widely accepted.

2.2.1 The fluid-mosaic model of membrane structure

According to Singer and Nicolson's **fluid-mosaic model**, the basic structure of the membrane is a fluid lipid bilayer with membrane proteins that are either embedded in this bilayer (integral proteins), in some cases spanning it, or attached to it (peripheral proteins), as illustrated for a cell membrane in Figure 2.4. These proteins are described further in Section 2.4.2.

Key to this model is the idea that the lipids and proteins of the membrane are highly mobile in the plane of the membrane; though they do not, as you will see later, readily cross between the two sides of the bilayer, termed **leaflets**. In cell membranes, many lipids and proteins have covalently attached carbohydrate groups which are presented at the extracellular surface, accounting for the carbohydrate content detailed in Table 2.2. These covalently attached carbohydrate groups form what is known as the **glycocalyx**, a tangled layer on the outside of the cell which has an important role in cell–cell recognition and adhesion.

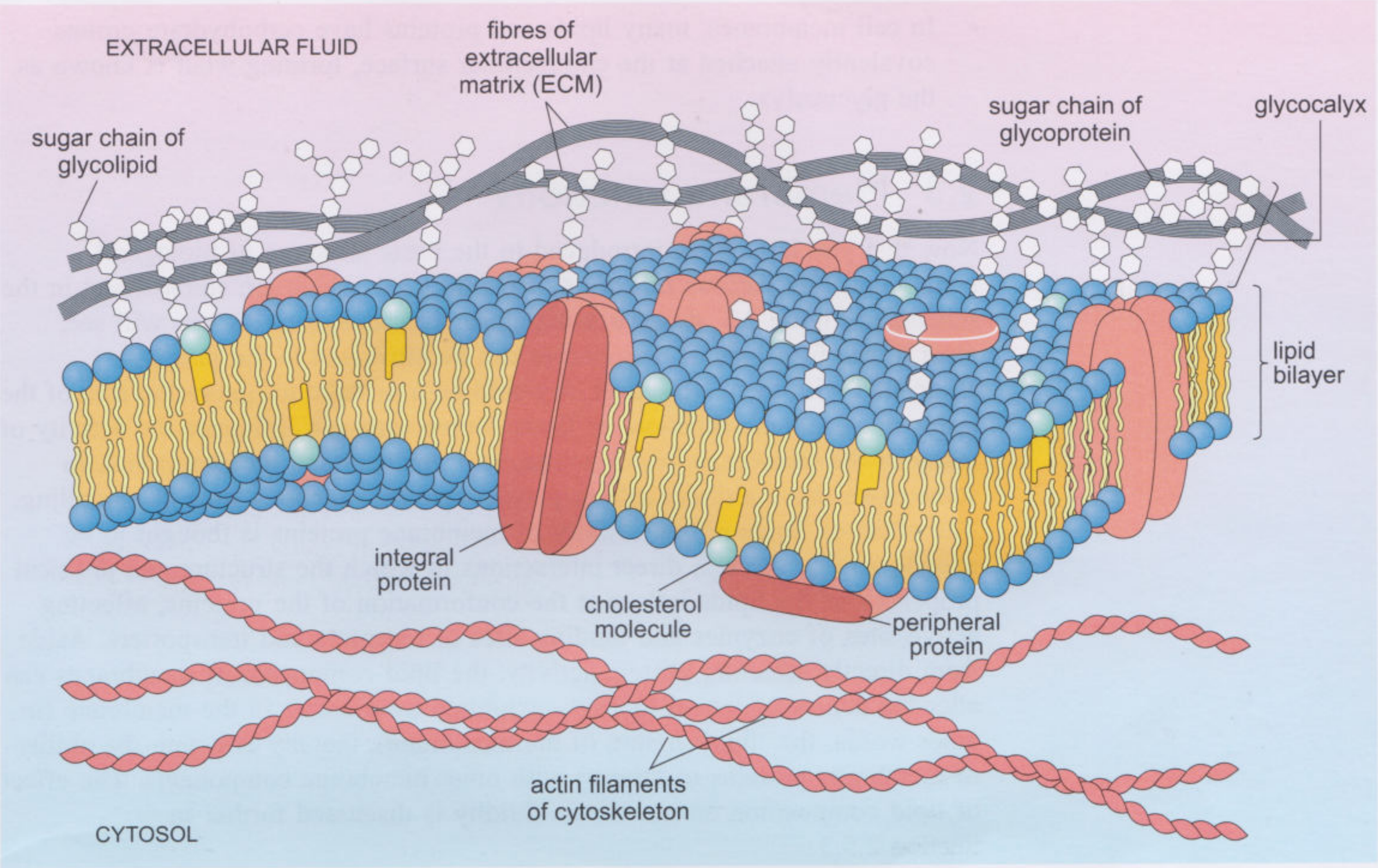

EXTRACELLULAR FLUID
fibres of extracellular matrix (ECM)
sugar chain of glycolipid
sugar chain of glycoprotein
glycocalyx
lipid bilayer
integral protein
cholesterol molecule
peripheral protein
actin filaments of cytoskeleton
CYTOSOL

Figure 2.4 Schematic of a cell membrane illustrating the fluid-mosaic model of membrane structure, as described by Singer and Nicolson in 1972. A fluid lipid bilayer gives the membrane its structure and membrane proteins move around in the plane of the membrane. On the extracellular surface of the cell membrane, as shown here, some of the lipids and proteins have covalently attached carbohydrate groups composed of sugar residues (forming what is known as the glycocalyx); note that the same is true of the luminal (lumen-facing) side of the endoplasmic reticulum and Golgi apparatus.

Summary of Section 2.2

- Biological membranes are composed of lipids, proteins and carbohydrates. Their specific molecular composition is directly related to their physical properties and so to their function.
- The major lipid components are phospholipids, which, like all membrane lipids, are amphipathic and spontaneously form a bilayer structure with the hydrophilic heads facing outwards and the hydrophobic tails projecting towards the interior of the bilayer.
- The preparation of artificial membranes (e.g. liposomes) has facilitated a great deal of research on the structure, properties and functions of lipid bilayers.
- In the fluid-mosaic model of membrane structure, membrane proteins are associated with a lipid bilayer, in some cases spanning it, and the lipids and proteins are highly mobile in the plane of the membrane.

- In cell membranes, many lipids and proteins have carbohydrate groups covalently attached at the extracellular surface, forming what is known as the glycocalyx.

2.3 Membrane functions

Now that you have been introduced to the basic structure of biological membranes, the various functions of membranes, which are summarised in the schematic Figure 2.5, deserve some closer consideration. As you will see, most of the functions of membranes can be attributed to the proteins associated with them. However, it would be a mistake to ignore the role of the lipids in membrane function, as these components can influence the activity of many of the membrane proteins (enzymes, transporters, receptors) and, in some cases, have a direct role to play in cellular functions such as signalling. The effect of lipids on the activity of membrane proteins is thought to be exerted largely through direct interactions in which the structure and physical properties of the lipids influence the conformation of the proteins, affecting active sites of enzymes and binding sites of receptors and transporters. Aside from directly affecting protein activity, the lipid component of membranes can alter the degree of lateral mobility exhibited by proteins in the membrane (in other words, the 'fluid' nature of the membrane), thereby affecting the ability of membrane proteins to interact with other membrane components. The effect of lipid composition on membrane fluidity is discussed further in Section 2.5.3.

2.3.1 Defining boundaries and serving as a permeability barrier

As has already been mentioned, the cell membrane is a physical barrier that separates the cell contents from the surrounding environment and regulates the passage of solutes both into and out of the cell. In eukaryotic cells, intracellular membranes similarly provide a boundary between the cytosol and the contents of the organelle.

Because of its hydrophobic interior, the lipid bilayer is not freely permeable to charged or polar (hydrophilic) solutes such as ions, amino acids or glucose. In contrast, small non-polar molecules such as carbon dioxide and oxygen can cross it easily. However, as you will learn later in this chapter, though the lipid bilayer provides a barrier to free movement of hydrophilic solutes, such substances do in fact cross biological membranes as a result of the activities of a variety of specific transport proteins.

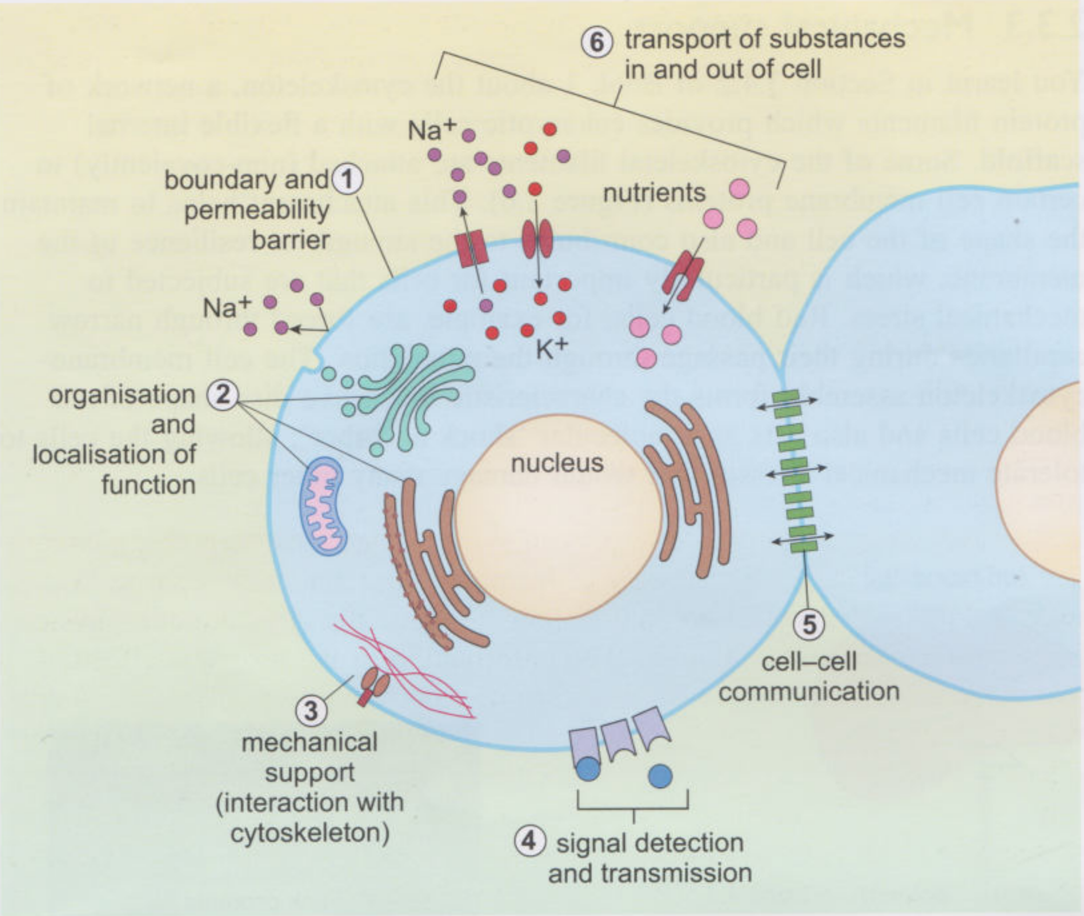

⑥ transport of substances in and out of cell
Na^+
boundary and ① permeability barrier
nutrients
Na^+
K^+
organisation ② and localisation of function
nucleus
⑤ cell–cell communication
③ mechanical support (interaction with cytoskeleton)
④ signal detection and transmission

Figure 2.5 Schematic diagram showing the various functions of membranes in the cell. (1) Defining the boundaries of the cell and its organelles and serving as a selective permeability barrier to the free movement of solutes. (2) Localising specific functions to distinct organelles or membranes. (3) Providing mechanical support by connecting with the cytoskeleton. (4) As the site of transmission and detection of signals. (5) Providing direct contact and cell–cell communication with neighbouring cells in tissues. (6) Facilitating and regulating transport of substances into and out of the cell.

2.3.2 Localisation of specific functions

Membranes differ in their chemical composition and, particularly, in the profile of proteins that they contain or that are associated with them. Indeed, membrane proteins are often used to characterise different membranes or identify the specific cell type or organelle to which they belong, or even to distinguish between different membranes in the same organelle. Crucially, the protein composition of membranes reflects their specific functions. For example, the inner mitochondrial membrane contains a complement of proteins that is quite distinct from the outer mitochondrial membrane, and indeed from any other membrane in eukaryotic cells. The distinctive protein components of the inner mitochondrial membrane include those that are involved in generating ATP for the cell by a process known as oxidative phosphorylation (discussed in detail in the following chapter). Another example of how specific functions are associated with membranes through localisation of particular proteins is the presence of the enzymes required to synthesise the components of the cell wall in the cell membrane of bacteria, plants and fungi.

2.3.3 Mechanical support

You learnt in Section 3.4.2 of Book 1 about the cytoskeleton, a network of protein filaments which provides eukaryotic cells with a flexible internal scaffold. Some of the cytoskeletal filaments are attached (non-covalently) to certain cell membrane proteins (Figure 2.6). This attachment helps to maintain the shape of the cell and also contributes to the strength or resilience of the membrane, which is particularly important for cells that are subjected to mechanical stress. Red blood cells, for example, are forced through narrow capillaries during their passage through the circulation. The cell membrane–cytoskeleton assembly forms the characteristic biconcave disc shape of red blood cells and also acts as a molecular 'shock absorber', allowing the cells to tolerate mechanical stresses that would damage many other cells.

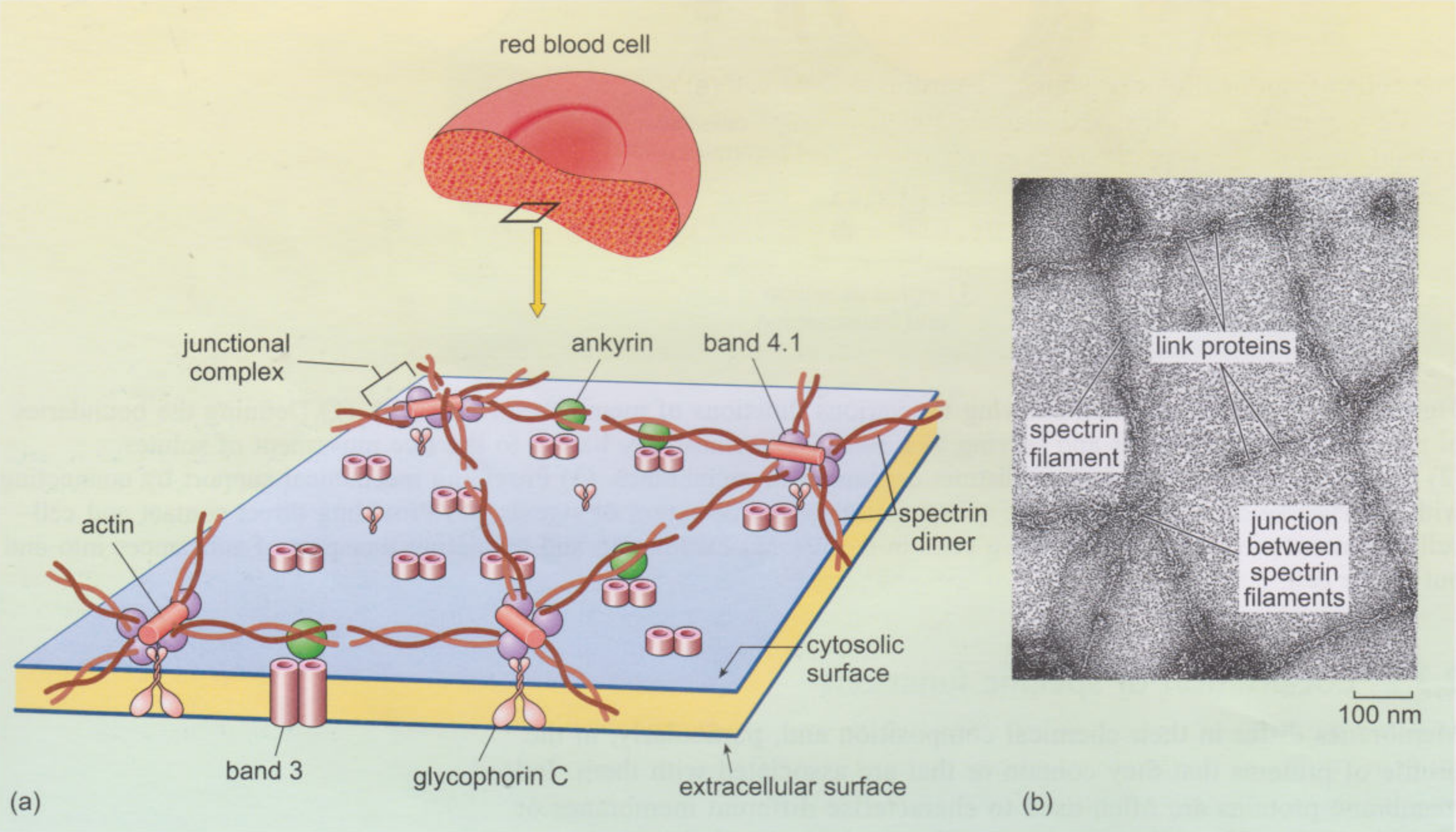


Figure 2.6 (a) Diagram of a red blood cell showing the relationship between the inner surface of the membrane and the cytoskeleton. Notice the two different kinds of link proteins (ankyrin and band 4.1) which attach two different membrane proteins (band 3 and glycophorin C, respectively) to the spectrin filaments. The band 4.1–glycophorin–spectrin interaction forms what is known as a junctional complex, which is, in turn, associated with actin microfilaments of the cytoskeleton. Note that band 3 and band 4.1 proteins owe their names to their original identification as bands in an electrophoretic analysis of erythrocyte membranes. (b) Electron micrograph showing spectrin filaments in red cell ghosts.

Spectrin is a component of the cytoskeleton that cross-links actin filaments. Filaments of spectrin can be seen associated with membranes in EM photos of red cell 'ghosts' (what remains of the cells after rupturing them to release their contents); hence the name of this protein (from spectre). When spectrin is dissociated from the ghosts, the membrane breaks up into small, spherical vesicles, demonstrating the importance of the cytoskeletal association to the integrity of the red blood cell membrane.

- ■ Why would people who produce abnormal spectrin have spherical red blood cells and be anaemic?
- □ Without functional spectrin, the correct cell shape cannot be maintained and, because the unsupported cell membrane is fragile, the cells are easily ruptured and destroyed. Anaemia results if red blood cells are destroyed faster than they can be replaced.

A similar network of spectrin-like filaments is found associated with the cell membrane in other eukaryotic cells too, but their organisation and functions are less well understood than they are in red blood cells.

2.3.4 Transmission and detection of signals

The cells of multicellular organisms coordinate their activities by exchanging a wide variety of chemical and electrical signals. Intercellular communication depends on processes that take place at or within the cell membrane and signals are communicated or transmitted from the exterior of the cell to inside the cell by a process known as signal transduction. Very little will be said about this here because Chapter 4 is devoted to the topic of communication between cells.

Figure 2.7 shows the two routes by which extracellular signals are received by the cell. A few hormones (e.g. the steroid sex hormones in vertebrates) are hydrophobic and lipid-soluble and so enter the cell directly by diffusing through the lipid bilayer. Once inside the cell, such signalling molecules then bind to their intracellular receptors and the signal-receptor complex translocates to the nucleus where it directly affects gene expression. In contrast, hydrophilic signalling molecules cannot pass unaided through the lipid bilayer, but bind to the extracellular domains of specific receptor proteins in the cell membrane. Binding of the ligand molecule (i.e. the signalling molecule) causes a change in the receptor protein, which results in a response. In many cases (e.g. with ligands called chemokines, which are capable of inducing directed movement or migration of responsive cells), the response may be the activation of a particular enzyme, commonly one at the beginning of a sequence of reactions. In other cases, the receptor may be a channel protein, i.e. an integral membrane protein that forms a channel through which a particular ion may pass. In such cases, binding of the signalling molecule causes the channel to open and allows passive entry of the ion. Ion channel-linked receptors include those for neurotransmitters (such as glutamate and acetylcholine), which are responsible for excitation of neurons. The intracellular events that occur as a result of binding of a signalling molecule to a cell-surface receptor can be numerous and varied and may ultimately result in changes in gene expression, as represented in Figure 2.7.

Steroids are a family of chemically related organic molecules, containing a characteristic four-ring structure. They include certain vertebrate sex hormones, such as testosterone and oestrogen, and glucocorticoids such as cortisol. Cholesterol, a lipid component of membranes, and also an important nutrient, is a steroid. Note, however, that cholesterol is not a steroid hormone.

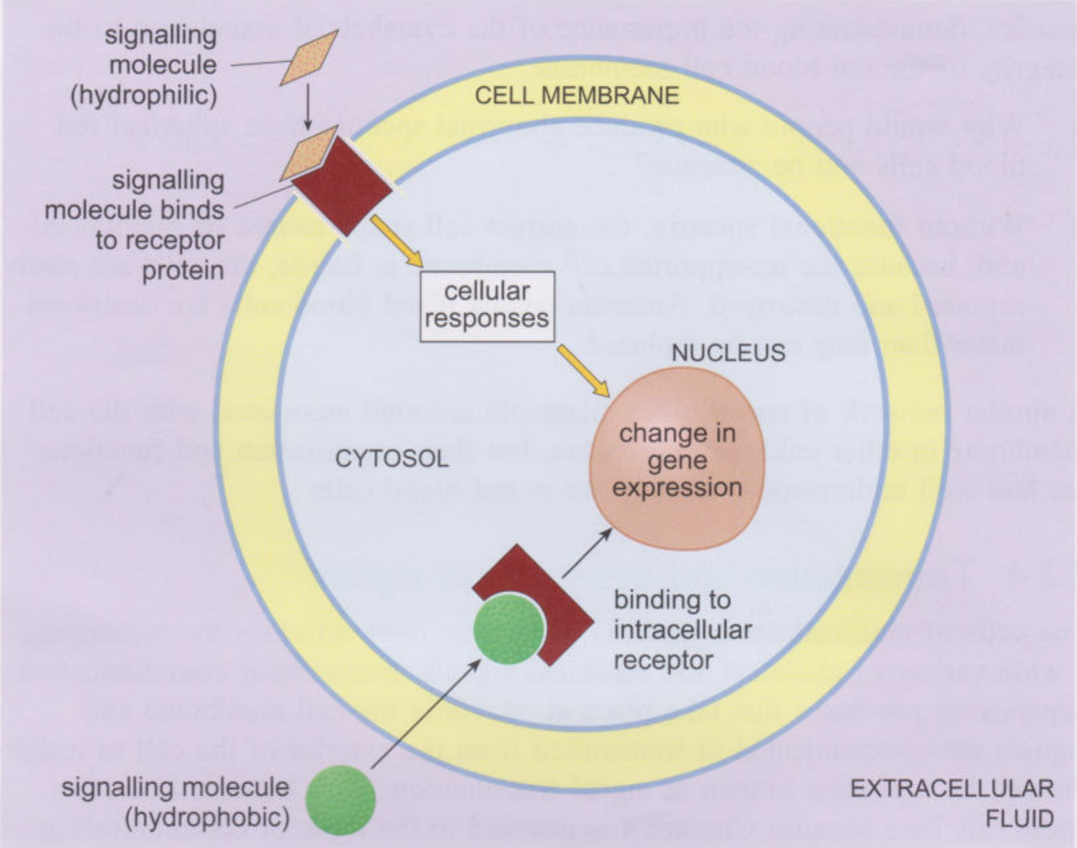

signalling molecule (hydrophilic)
CELL MEMBRANE
signalling molecule binds to receptor protein
cellular responses
NUCLEUS
change in gene expression
CYTOSOL
binding to intracellular receptor
signalling molecule (hydrophobic)
EXTRACELLULAR FLUID

Figure 2.7 Membrane-bound and intracellular receptors. Hydrophobic signalling molecules pass unaided through the membrane and bind to their receptors in the cytosol (or nucleus). Hydrophilic signalling molecules bind to a receptor protein on the membrane surface.

2.3.5 Cell–cell communication, recognition and adhesion

The cell membrane is the site at which all intercellular interactions take place – between adjacent cells in multicellular organisms, between cells and the extracellular matrix, or between cells and pathogens (including bacteria and viruses). The ability of cells to recognise and adhere to each other or to the extracellular matrix is essential for the maintenance of tissues and is particularly important during development in many organisms. Interactions between specific membrane proteins on adjacent cells create stable distinct regions of contact, known as junctions. Such junctions not only link neighbouring cells but, in some cases, anchor cells to the surrounding extracellular matrix. Intercellular junctions were described in Chapter 3 of Book 1.

- ■ What are the four types of junction that are found between cells?
- □ There are four structurally and functionally distinct types of cell junction: tight junctions, anchoring junctions, adherens junctions and gap junctions (Book 1, Section 3.5.2).

During the course of embryonic development in animals, cells have to group together (with the right neighbours), in the right place and at the right time. Before the various types of junction characteristic of mature tissues become fully functional, the appropriate cell–cell recognition and adhesion processes have to occur. As well as forming stable interactions, dynamic cell–cell and cell–matrix interactions are also important in migration of cells within tissues or, in vertebrates, in the traffic of cells between the blood and surrounding tissues. Selective cell–cell and cell–matrix recognition and adhesion are mediated by specific non-covalent interactions between members of a variety of transmembrane (membrane-spanning) glycoprotein families (Table 2.1).

Table 2.1 The major transmembrane glycoproteins involved in cell–cell recognition and adhesion. Some of the interactions contribute to stable junctions (indicated where appropriate).

Transmembrane glycoprotein	Binds to...	Type of junction
cadherins	cadherins in cell membrane of adherent cell	adherens and anchoring junctions (desmosomes)
cell adhesion molecules (CAMs)	integrins and CAMs in the cell membrane of adherent cell	
selectins	carbohydrate groups on glycoproteins in the cell membrane of adherent cell	
integrins	CAMs in cell membrane of adherent cell	
	extracellular matrix proteins	anchoring junctions (hemidesmosomes)
connexins	connexins in cell membrane of adherent cell	gap junctions
claudins	claudins in cell membrane of adherent cell	tight junctions
occludins	occludins in cell membrane of adherent cell	tight junctions

Gap junctions consist of clusters of membrane protein structures known as *connexons* (Figure 2.8). Connexons are assemblies of six connexin protein subunits that span the membrane and are aligned with connexons in the membrane of the adjacent cell, creating 1.5 nm diameter 'tunnels' connecting the cytosolic compartments of the cells. These channels are big enough to allow passage of molecules of M_r less than 1000, including sugars, amino acids and small peptides, but not proteins or nucleic acids.

Unlike gap junctions, the other types of cell junction do not permit direct electrical or chemical communication between cells. Instead they are purely adhesive junctions (adherens junctions, anchoring junctions known as desmosomes and tight junctions) between adjacent cells, or between a cell and components of the extracellular matrix (e.g. another type of anchoring junction known as hemidesmosomes). Inside the cell, the protein components of these junctions can interact with other proteins, known as intracellular attachment proteins, which in turn can interact with proteins of the cytoskeleton. In this way, there is a connection, via such junctions, between the cytoskeletons of adjacent cells, or between a cell's cytoskeleton and the extracellular matrix.

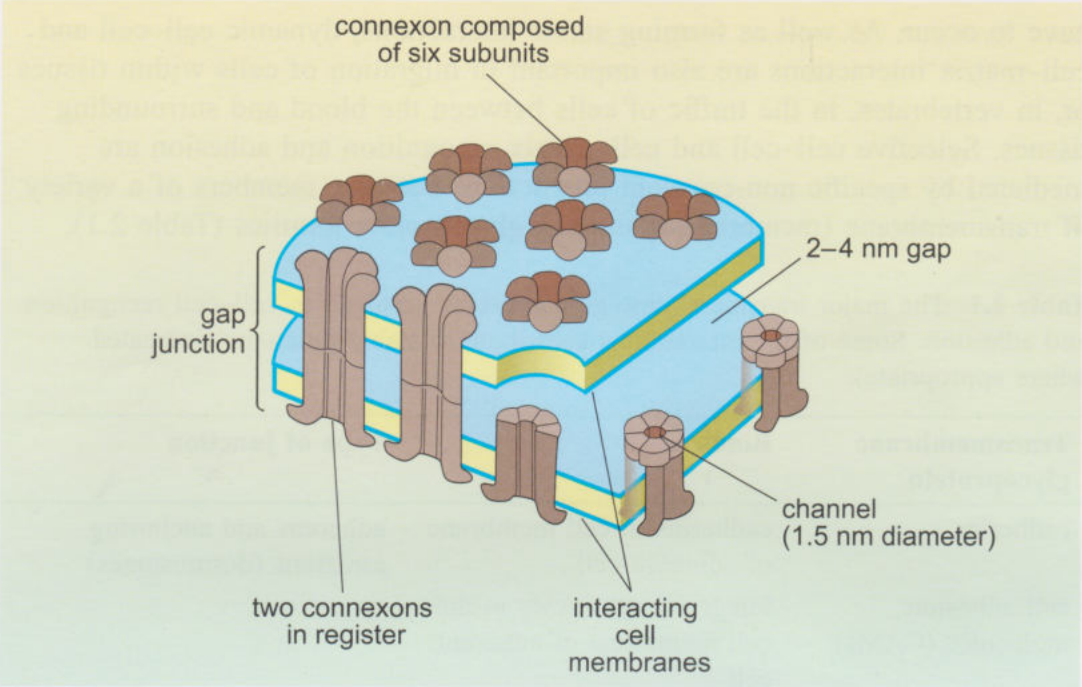

connexon composed of six subunits
2–4 nm gap
gap junction
channel (1.5 nm diameter)
two connexons in register
interacting cell membranes

Figure 2.8 Connexons consist of an assembly of six connexin subunits in the membranes of adjacent cells, which together form a central channel that links the cytosolic compartments of the two cells.

Such interactions enable cells in tissues to function as a unit and are particularly important in how tissues resist and respond to physical stresses and strains.

Many of the cadherins and cell adhesion molecules (Table 2.1) form what are known as *homophilic* interactions, i.e. they bind to identical molecules on the surface of the cell to which they adhere. In contrast, the selectins and integrins bind with different molecules in the adherent cell, establishing *heterophilic* interactions.

■ The sugar components of many of the transmembrane glycoproteins involved in cell–cell adhesion are negatively charged (because they contain acidic sugar residues). How would the presence of chains of negatively charged sugars on adhesion molecules affect adhesion between neighbouring cells?

□ The negatively charged sugar chains would cause adhesion molecules on adjacent cells to repel each other and so reduce cell adhesion.

The degree of adhesion between cells depends in part on the nature and extent of glycosylation of the adhesion molecules; the fewer negatively charged sugar residues, the stronger the adhesion. In fact, alterations in CAM glycosylation are believed to be important mechanisms by which cell–cell interactions are regulated in embryonic development.

2.3.6 Transport and its regulation

To ensure that intracellular conditions are optimal for cellular processes, the transfer of materials across the cell membrane is selective and controlled. For example, metabolic processes require entry of nutrients (and usually oxygen) and the exit of waste products. Similarly, response to some external signals requires the passage of the signalling molecule into the cell or involves specific, transitory ion movements across the cell membrane. In addition, cells may secrete molecules (e.g. extracellular enzymes, hormones) into the external medium or take up large molecules or particles. Controlled transfer of specific molecules and ions across intracellular membranes also occurs and is important in the movement of materials between intracellular compartments.

Many membrane proteins are involved in transport processes directly or in their regulation. Generally, each transport protein recognises and transports a specific solute, or a group of related molecules. The subject of transport across cell membranes is discussed in some detail in Sections 2.8 and 2.9.

Summary of Section 2.3

- The protein composition of membranes reflects their specific functions.
- The cell membrane separates the cell contents from the surrounding environment and regulates the passage of solutes both into and out of the cell. In eukaryotic cells, intracellular membranes separate the cytosol from the contents of organelles.
- The association of cell membrane proteins with cytoskeletal components provides mechanical support to the cell.
- Intercellular communication depends on processes that take place at or within the cell membrane. Some extracellular signalling molecules are hydrophobic and can enter the cell directly by diffusing through the lipid bilayer before binding to their intracellular receptors. Other signalling molecules bind to the extracellular domains of receptor proteins in the cell membrane, thereby triggering a series of events that ultimately leads to a response.
- The cell membrane is the site at which all intercellular interactions take place. Interactions between specific membrane proteins on adjacent cells create stable distinct regions of contact, known as junctions.
- Selective cell–cell and cell–matrix recognition and adhesion are mediated by specific non-covalent interactions between members of a variety of transmembrane glycoprotein families including cadherins, cell adhesion molecules, selectins, integrins, connexins, claudins and occludins.

2.4 Membrane composition

Chemical composition varies greatly between membranes in different cell types and, in eukaryotic cells, between different membranes within a single cell. Table 2.2 details the relative content by mass of the three biochemical components in a variety of membranes. In most membranes, protein content (percentage by mass) exceeds that of lipid; however, protein molecules are, on average, about 50 times larger than lipid molecules, so in fact membrane lipid molecules always far outnumber the proteins present.

Table 2.2 Protein, lipid and carbohydrate content (approximate percentage by mass) and protein to lipid ratio of biological membranes. (Adapted from Becker et al., 2009)

Membrane		Approximate % by mass			Protein/ lipid ratio
		Protein	Lipid	Carbohydrate	
cell membranes	human red blood cell	49	43	8	1.14
	mammalian liver cell	54	36	10	1.50
	amoeba	54	42	4	1.29
	Gram-positive bacterium	75	25	0	3.00
intracellular membranes	nuclear envelope	66	32	2	2.06
	endoplasmic reticulum	63	27	10	2.33
	Golgi apparatus	64	26	10	2.46
	chloroplast lamella	70	30	0	2.33
	mitochondrial inner membrane	78	22	0	3.54
	mitochondrial outer membrane	55	45	0	1.22

■ From the data provided in Table 2.2, what do you notice about the composition of the bacterial membrane, the mitochondrial membranes and the chloroplast lamella?

□ None of these membranes contains carbohydrate.

■ Bearing in mind what you learnt about the evolution of organelles in Chapter 1 of Book 1, comment on this observation.

□ According to the endosymbiotic theory, mitochondria and chloroplasts are thought to have originated from prokaryotes that were internalised by, and established a symbiotic relationship with, other prokaryotic cells (Book 1, Section 1.2.4). Over time these cells evolved into eukaryotic cells containing mitochondria and chloroplasts.

■ In the light of what you have learnt about the function of mitochondria (Book 1, Section 3.4.10), comment on the relative protein content of the inner and outer mitochondrial membranes.

□ The inner mitochondrial membrane has a higher protein to lipid ratio than does the outer mitochondrial membrane. This reflects the role of the inner membrane in ATP synthesis, with the proteins involved contributing to its large protein content.

Aside from the relative amounts of lipid, protein and carbohydrate, the nature of each of these components also varies. The functions of membranes depend on their physical and chemical properties; so to explore the structure–function relationship of membranes more closely, it is necessary to consider the properties of their molecular components.

2.4.1 Membrane lipids

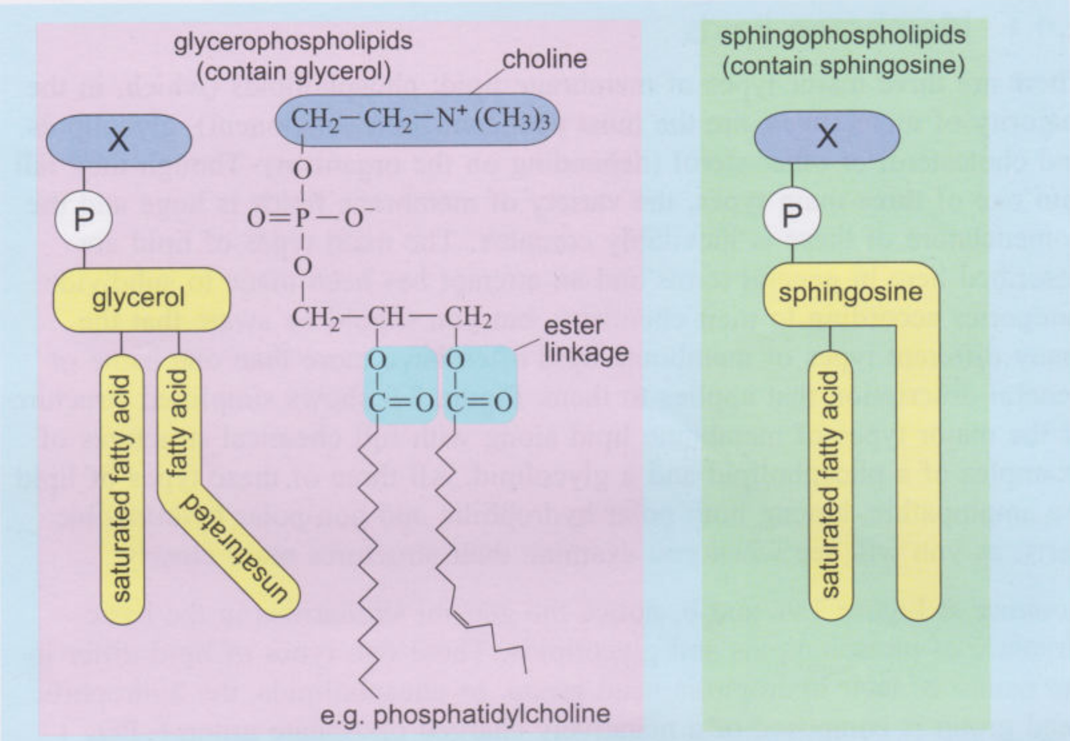
There are three major types of membrane lipid: phospholipids (which, in the majority of membranes, are the most abundant lipid component), glycolipids, and cholesterol or other sterol (depending on the organism). Though they fall into one of three main types, the variety of membrane lipids is huge and the nomenclature of these is inevitably complex. The main types of lipid are described here in general terms and an attempt has been made to subdivide categories according to their chemistry, but you should be aware that the many different types of membrane lipid often have more than one name or general description that applies to them. Figure 2.9 shows simplified structures of the major types of membrane lipid along with full chemical structures of examples of a phospholipid and a glycolipid. All three of these types of lipid are amphipathic, having both polar hydrophilic and non-polar hydrophobic parts, as you will see when you examine their structures more closely.

Looking at Figure 2.9a and b, notice the general similarities in the basic structure of phospholipids and glycolipids. These two types of lipid differ in the nature of their hydrophilic head group. In phospholipids, the hydrophilic head group is composed of a negatively charged phosphate group ($-PO_4^-$) attached to one of a number of other different polar groups (represented by X in Figure 2.9) Among the most significant membrane phospholipids are phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI). Derivatives of PI are involved in cell signalling, which you will learn about in Chapter 4. In glycolipids, the hydrophilic head group is a sugar residue (galactose or glucose), though in some glycolipids the carbohydrate component consists of many sugar residues linked together in a branching chain.

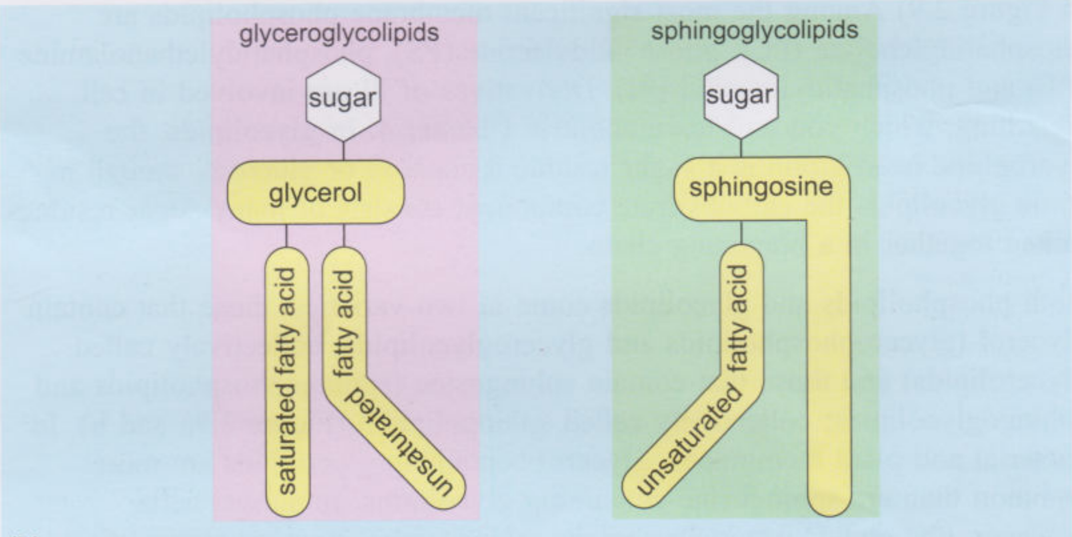
Both phospholipids and glycolipids come in two varieties: those that contain glycerol (glycerophospholipids and glyceroglycolipids; collectively called glycerolipids) and those that contain sphingosine (sphingophospholipids and sphingoglycolipids; collectively called sphingolipids) (Figure 2.9a and b). In bacterial and plant membranes, glycerol-containing glycolipids are more common than are sphingosine-containing glycolipids. In animal cells, however, glycolipids generally contain sphingosine. The proportions of particular types of lipid can vary between different types of membrane within an animal cell. For example, the concentration of sphingolipids is higher in ER membranes than in Golgi, and is highest in the cell membrane.

Many membrane glycolipids in animals are involved in cell recognition by the immune system (e.g. the ABO blood group determinants on red blood cells).

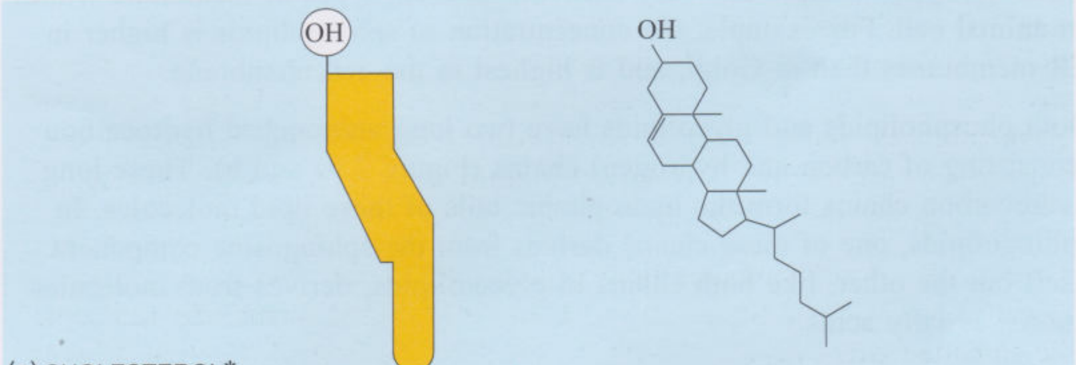
Both phospholipids and glycolipids have two long unbranched hydrocarbon (consisting of carbon and hydrogen) chains (Figure 2.9a and b). These long hydrocarbon chains form the hydrophobic tails of these lipid molecules. In sphingolipids, one of these chains derives from the sphingosine component itself but the other, like both chains in glycerolipids, derives from molecules known as fatty acids.



(a) PHOSPHOLIPIDS



(b) GLYCOLIPIDS

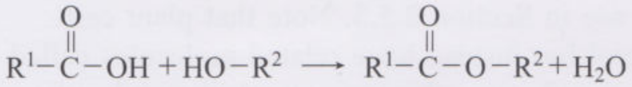


(c) CHOLESTEROL*

*Cholesterol is found in animal cell membranes. Plant cell membranes contain phytosterols, and prokaryote membranes contain sterol-like hopanoids.

Figure 2.9 Schematic representations of the major types of membrane lipid: (a) phospholipids; (b) glycolipids; and (c) cholesterol. Lipids that contain glycerol (glycerophospholipids and glyceroglycolipids) are collectively known as glycerolipids, while those that contain sphingosine (sphingophospholipids and sphingoglycolipids) are collectively known as sphingolipids. In (a), X represents a polar group of which the three most common are ethanolamine, choline and inositol. Note that all the membrane lipids are amphipathic, having both a hydrophobic and a hydrophilic component. A chemical structure is shown for a glycerophospholipid (in this case one containing a choline head group, a phosphatidylcholine) and for cholesterol.

Fatty acids are long hydrocarbon chains with an acidic carboxyl (–COOH) group at one end which reacts, as represented below, with a hydroxyl (–OH) group on glycerol or sphingosine to form what is known as an ester linkage in the lipid molecule:



$$R^1-\overset{\overset{\displaystyle O}{\|}}{C}-OH+HO-R^2 \longrightarrow R^1-\overset{\overset{\displaystyle O}{\|}}{C}-O-R^2+H_2O$$

Once incorporated into the lipid molecule in this way, the fatty acids are referred to as fatty acyl chains.

The fatty acyl chains in phospholipids and glycolipids usually contain between 14 and 24 carbon atoms (due to the way in which they are synthesised, most fatty acids have an even number of carbon atoms). Carbon–carbon bonds, such as those in fatty acids, can be single bonds or double bonds, depending on the number of electrons that participate in the covalent bond. Where a fatty acid contains no double bonds, it is described as **saturated**; fatty acids containing one or more C=C double bonds are described as **unsaturated**. You may be familiar with these terms from the nutritional information labels on food packaging.

In animal and plant cells, over half the fatty acids incorporated in membrane lipids are unsaturated. As you will see in Section 2.5, the degree of unsaturation and the length of the fatty acyl components of membrane lipids greatly affect their physical properties and hence the physical properties of the membrane to which they belong. A good illustration of how lipids can influence membrane function is provided by the insulin receptor, which is an integral membrane protein. By feeding cultured cells with polyunsaturated fatty acids, it is possible to increase incorporation of these fatty acids in the cell membranes, and this has the effect of increasing insulin binding to the receptor. The same effect is observed in artificial membranes (liposomes, Figure 2.3) in which the insulin receptor has been incorporated.

In prokaryotes too, membrane lipid structure can confer special properties on the cell. Most bacteria have a cell wall outside the cell membrane and some (the so-called Gram-negative bacteria introduced in Book 1, Section 3.3) also have a second, outer membrane surrounding the cell wall. Abundant in the outer leaflet of the outer membrane are lipid–sugar chains called, not glycolipids, but lipopolysaccharides (LPS). These molecules stimulate a powerful immune response in mammals and other vertebrates, which is why infection by some Gram-negative pathogens can lead to inflammation and fever. Archaea have a distinctive type of membrane lipid in which each of the long hydrocarbon chains is branched and is directly joined to glycerol via an oxygen atom (in an ether linkage). This contrasts with the linear hydrocarbon chains linked via an ester linkage that are characteristic of the membrane lipids of the Bacteria and all eukaryotes.

The third type of membrane lipid, **cholesterol** (Figure 2.9c), is structurally very different from the phospholipids and glycolipids, though it too is amphipathic. Cholesterol is a sterol which is a type of steroid, a group of molecules that are distinguished by a common four-ring chemical structure. In the case of cholesterol, there is a short hydrocarbon chain at one end of the four-ring structure and a weakly polar hydroxyl (–OH) group at the other. Cholesterol is a significant component of animal cell membranes (representing nearly 25% by mass of red blood cell membrane lipids), but constitutes a relatively minor proportion of the lipids of intracellular membranes. The amount of cholesterol in membranes can have a significant effect on membrane fluidity, as you will see in Section 2.5.3. Note that plant cell membranes don't have cholesterol but instead have related molecules called phytosterols, and in prokaryote membranes, there are sterol-like molecules called hopanoids.

The lipid components of membranes can be analysed using a technique known as thin-layer chromatography (described in Box 2.2).

Box 2.2 Analysing membrane lipids

Non-polar hydrophobic molecules are not readily soluble in aqueous (water-based) solvents. This is because water is a polar molecule, having as it does an asymmetric distribution of charge (Section 1.1.1 of Book 1). A general rule of solubility is that 'like dissolves like'. Thus polar (hydrophilic) substances such as amino acids are soluble in polar solvents such as water, while non-polar (hydrophobic) molecules are soluble in non-polar solvents such as chloroform or diethyl ether. The behaviour of amphipathic lipids in an aqueous medium was described in Box 2.1 and is responsible for the lipid bilayer structure characteristic of biological membranes.

Because of their relatively large hydrophobic components, membrane lipids are most readily dissolved in non-polar or weakly polar organic solvents. Essentially, the relative solubility of membrane lipids in different solvents depends on the relative sizes of their hydrophobic and hydrophilic portions and on the polarity of the latter (i.e. charged groups are more polar than, for example, the weakly polar hydroxyl group on cholesterol).

The solubility of lipids in organic solvents can be exploited to separate these compounds from other biological material. Following extraction with organic solvent, a number of different chromatography techniques can be applied to separate lipids. In thin-layer chromatography (TLC), lipids are resolved (i.e. separated from each other) on the basis of their relative affinities for a hydrophilic stationary phase and a hydrophobic mobile phase (the solvent) (Figure 2.10). The stationary phase is usually a thin layer of silica gel on a glass or metal plate onto which a sample of the lipid extract is spotted (at the origin). The organic solvent used to extract the lipids is allowed to evaporate and the edge of the plate is dipped into the mobile phase, which is usually a mixture of appropriate solvents (such as chloroform, methanol and water). The solvent moves up the plate by capillary action (as a result of its attraction to the solid stationary phase), and, as it passes the origin, it carries the lipids with it. The lipids travel at different rates according to their polarity. Weakly polar lipids such as cholesterol have little affinity for the hydrophilic stationary phase and therefore move quickly up the plate with the solvent. More polar lipids such as glycerophospholipids move less quickly as they have a greater affinity for the stationary phase. When the solvent front has nearly reached the top of the plate, the plate is removed from the reservoir containing the mobile phase, and dried. The lipids can be visualised using a chemically reactive spray. Running purified lipid preparations as standards can help to identify the lipids in the original mixture. By extracting lipid spots with, for example, chloroform, the lipid can be recovered for further analysis using more sophisticated chromatography techniques.

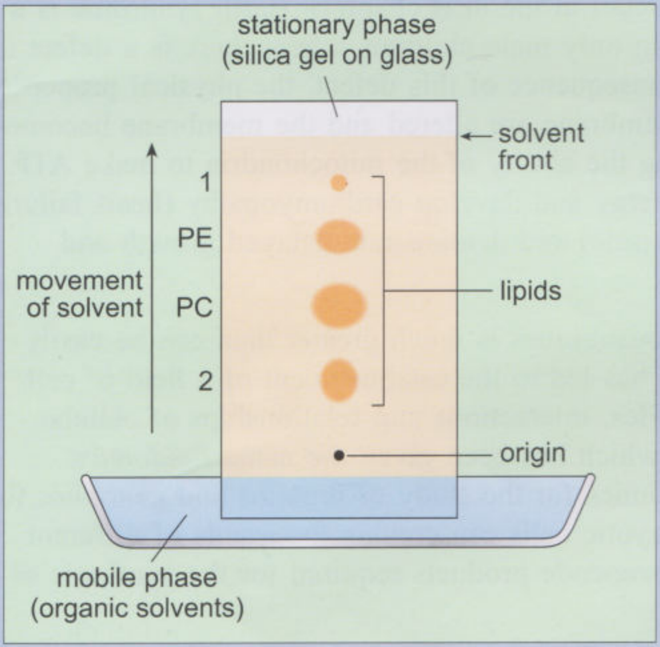


Figure 2.10 Analysis of membrane lipids by thin-layer chromatography (TLC). The lipid extract is spotted onto the edge of the TLC plate (stationary phase) and the plate is dipped into a solvent mixture (mobile phase). Weakly polar lipids move quickly up the plate, while strongly polar lipids move more slowly. The identity of the spots is discussed in the main text.

- Figure 2.10 shows the resolution of lipids from red blood cell membranes by TLC. Spots attributable to two phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC), have been identified. Considering their relative polarity, identify which of the other two spots (numbered 1 and 2 in the figure) corresponds to each of cholesterol and the phospholipid phosphatidylserine (PS).

☐ Polar lipids move more slowly up the hydrophilic stationary phase than do non-polar or weakly polar lipids; thus the lipid in spot 1 must be less polar than that in spot 2. Cholesterol has a weakly polar hydroxyl group, whereas phospholipids such as PS are more strongly polar, due to their charged head group; therefore spot 1 corresponds to cholesterol as the least polar lipid, and spot 2 corresponds to PS.

While the phospholipids, glycolipids and cholesterol described here are the major types of lipid found in cell membranes, there are many others present in smaller amounts or which are limited to particular specialised types of membrane, where they often play a particular role or influence the properties of the membrane in some significant way. An example is a phospholipid called cardiolipin, which has four fatty acyl chains, instead of the usual two. Cardiolipin is found in the mitochondrial inner membrane, where its four fatty acyl chains help it to make the membrane more stable and resistant to the huge electrical stress imposed by the activities of the electron transport chain (which will be discussed in detail in the next chapter). Barth syndrome is a rare genetic problem, affecting only male children, where there is a defect in cardiolipin synthesis. As a consequence of this defect, the physical properties of the inner mitochondrial membrane are altered and the membrane becomes leaky, seriously compromising the ability of the mitochondria to make ATP. The affected children lack energy and develop cardiomyopathy (heart failure and weakness of the heart muscle) and demonstrate delayed growth and skeletal muscle weakness.

The variety of lipids in cell membranes is much greater than can be easily conveyed in this chapter and has led to the establishment of a field of cell biology dedicated to the profiles, interactions and relationships of cellular lipids in biological systems, which has been given the name *lipidomics* (analogous to the term proteomics for the study of proteins and genomics for the study of genomes). Eukaryotic cells can contain thousands of different lipids and ~5% of their genes encode products required for the synthesis of lipids (van Meer et al., 2008).

2.4.2 Membrane proteins

Approximately 30% of the proteins encoded by the human genome are membrane proteins – evidence of their importance in cellular function. It is proteins that perform the specific functions of the membrane, as opposed to the more structural and physical role of the lipid bilayer. The diversity of membrane proteins is reflected in the variety of functions that they serve:

- transport of specific molecules and ions into or out of the cell or organelle (Section 2.8)
- receptors for chemical signals from outside the cell or organelle (to be discussed in Chapter 4)

- structural links between the cytoskeleton and the extracellular matrix (Sections 2.3.3 and 2.3.5)
- intercellular recognition and adhesion (Section 2.3.5)
- catalysis – the membranes of organelles are characterised by distinctive enzyme components.

Broadly speaking, membrane-associated proteins can be divided into three categories, integral, peripheral and lipid-linked, based on how they associate with the lipid bilayer (Figure 2.11).

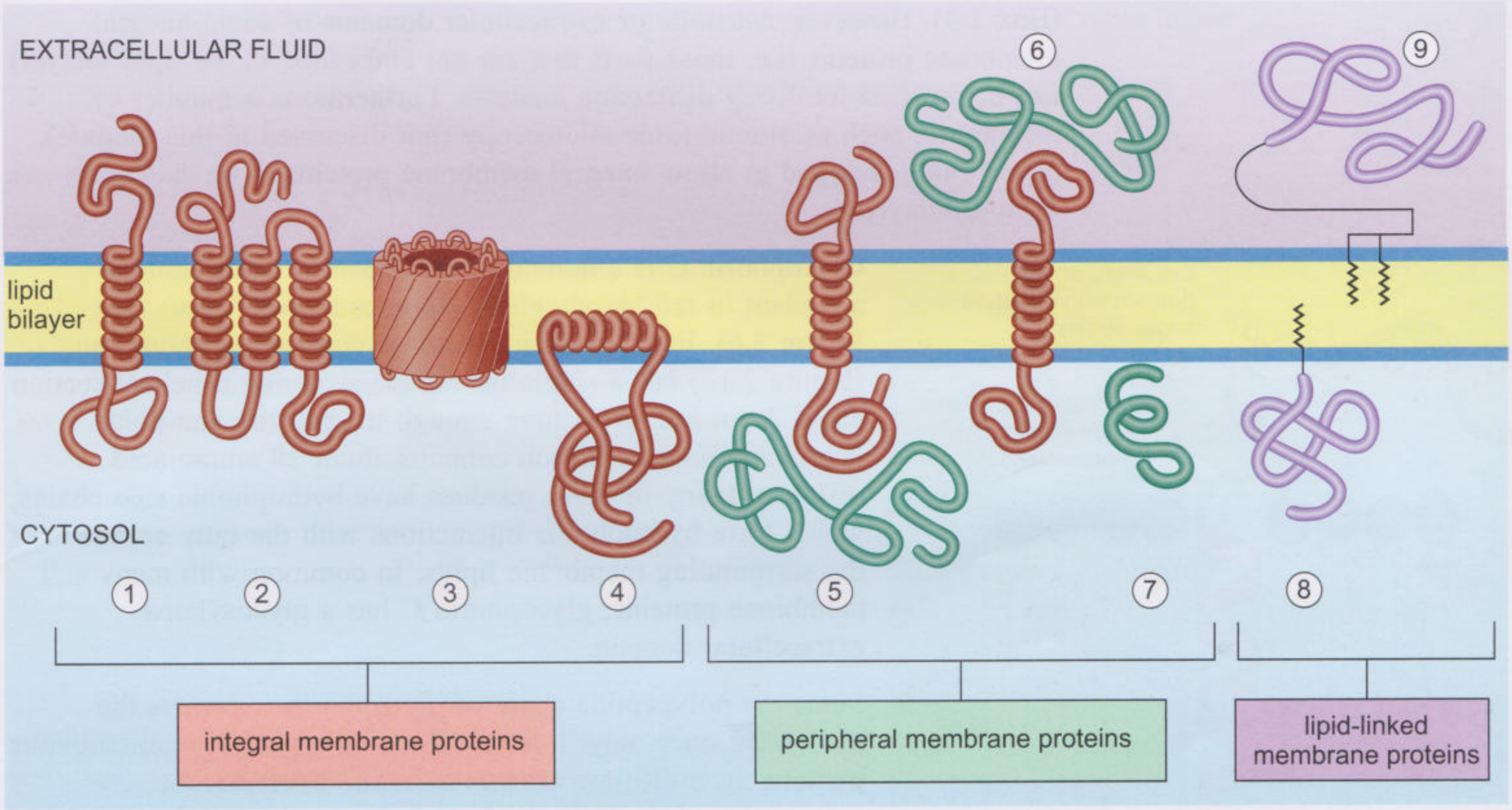

EXTRACELLULAR FLUID
lipid bilayer
CYTOSOL
①
②
③
④
⑤
⑥
⑦
⑧
⑨
integral membrane proteins
peripheral membrane proteins
lipid-linked membrane proteins

Figure 2.11 Membrane proteins fall into one of three categories. Integral membrane proteins (1–4) have some portion that interacts with the hydrophobic core of the lipid bilayer. Transmembrane proteins span the lipid bilayer (1–3) and a polypeptide chain can traverse the membrane once only (single-pass) or more than once (multipass). The membrane-spanning portions of these proteins can have α helical or β sheet structures, as described in the text. Other integral membrane proteins are embedded in the membrane on one side only (4). Peripheral proteins (5–7) are not associated with the hydrophobic interior of the lipid bilayer; instead, they either associate with the membrane indirectly via non-covalent interactions with other membrane proteins (5, 6) or directly via interactions with the hydrophilic head groups of the lipids (7). Lipid-linked membrane proteins (8, 9) are anchored in the bilayer by means of a lipid linkage at the cytosolic surface (8) or the extracellular surface (9).

Most membrane proteins are described as **integral**, having one or more portions that are hydrophobic, through which they associate with the hydrophobic interior of the lipid bilayer. Other portions of the protein are hydrophilic; thus integral membrane proteins are in fact amphipathic, just as the lipid components of membranes are. The polar portions of the protein associate with the polar head groups of the lipids via hydrogen bonding and ionic interactions, or are buried deep within the protein. Integral proteins can associate with the lipid bilayer in a number of different ways, illustrated in Figure 2.11. Most, called **transmembrane proteins**, span the lipid bilayer (proteins 1, 2 and 3 in Figure 2.11). Others are embedded at only one surface of the membrane, generally the cytosolic side, and are anchored in the membrane by a hydrophobic α helical segment (protein 4 in Figure 2.11).

Because of their affinity for the lipid bilayer, integral membrane proteins are difficult to isolate; and generally treatment with a detergent (to disrupt the lipid bilayer) is necessary in order to extract these proteins. Studying integral membrane proteins is also difficult as they need to be within the membrane to achieve their native conformation. Thus it is very difficult to crystallise them for X-ray diffraction studies of the kind described in the previous chapter (Box 1.3). However, cytosolic or extracellular domains of some integral membrane proteins (i.e. those parts that are not embedded in the lipid bilayer) can be purified for X-ray diffraction analysis. Furthermore, a number of techniques, such as atomic force microscopy (not discussed in this module), have been developed to allow integral membrane proteins to be studied *in situ* in lipid bilayers.

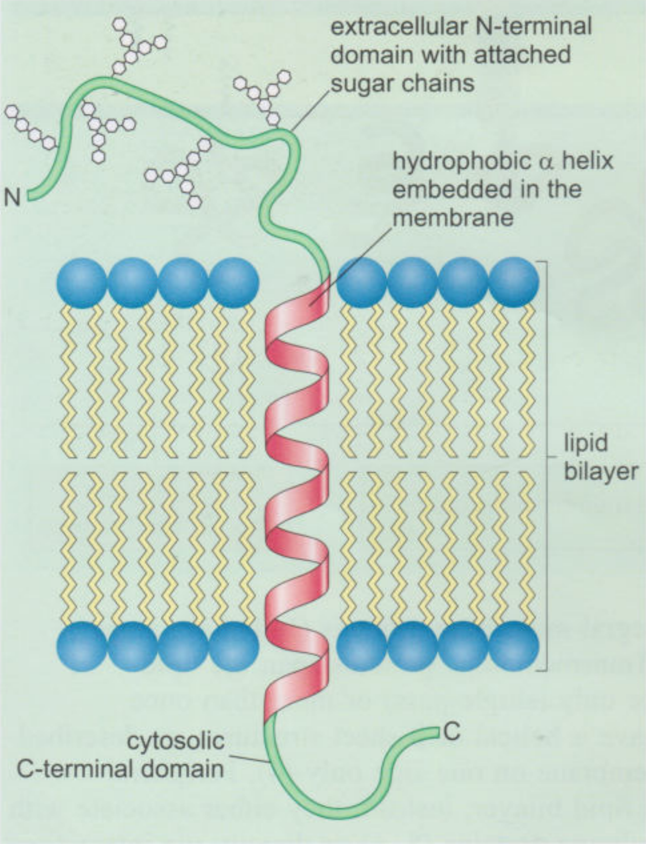

extracellular N-terminal domain with attached sugar chains
hydrophobic α helix embedded in the membrane
N
lipid bilayer
cytosolic C-terminal domain
C

Figure 2.12 Structure of a single glycophorin C subunit. Glycophorin C is a single-pass transmembrane protein (the polypeptide crosses the membrane once only), found in the cell membrane of vertebrate red blood cells. The extracellular domain is heavily glycosylated and the functional protein is a dimer.

Glycophorin C is a homodimeric transmembrane protein abundant in red blood cell membranes in vertebrates (see Figure 2.6). Each of the identical glycophorin C subunits (Figure 2.12) has a single membrane-spanning α helical section about 3 nm long (just long enough to span the non-polar membrane interior) which contains about 20 amino acid residues. Many of these residues have hydrophobic side chains, which form hydrophobic interactions with the fatty acyl tails of the surrounding membrane lipids. In common with many cell membrane proteins, glycophorin C has a glycosylated extracellular domain.

Since the polypeptide chain of glycophorin C crosses the membrane once only, it is called a **single-pass transmembrane protein**. In **multipass transmembrane proteins**, the polypeptide chain loops back and crosses the membrane several times. An example is bacteriorhodopsin, a protein that captures light energy and uses it to pump protons (H^+) across the membrane, providing energy for the cell (discussed further in Chapter 3). Bacteriorhodopsin is a component of the purple membrane of the archaeon (singular of Archaea), *Halobacterium halobium*, which is found in very salty aquatic environments. It is a member of a family of proteins known as 7-helix transmembrane receptors as it contains a bundle of seven α helices joined by short hydrophilic sections (Figure 2.13). In the case of bacteriorhodopsin, the seven membrane-spanning helices form a channel through which protons are pumped across the membrane. Other 7-helix transmembrane receptors have a critical role in signal transduction in other types of cell (see Chapter 4).

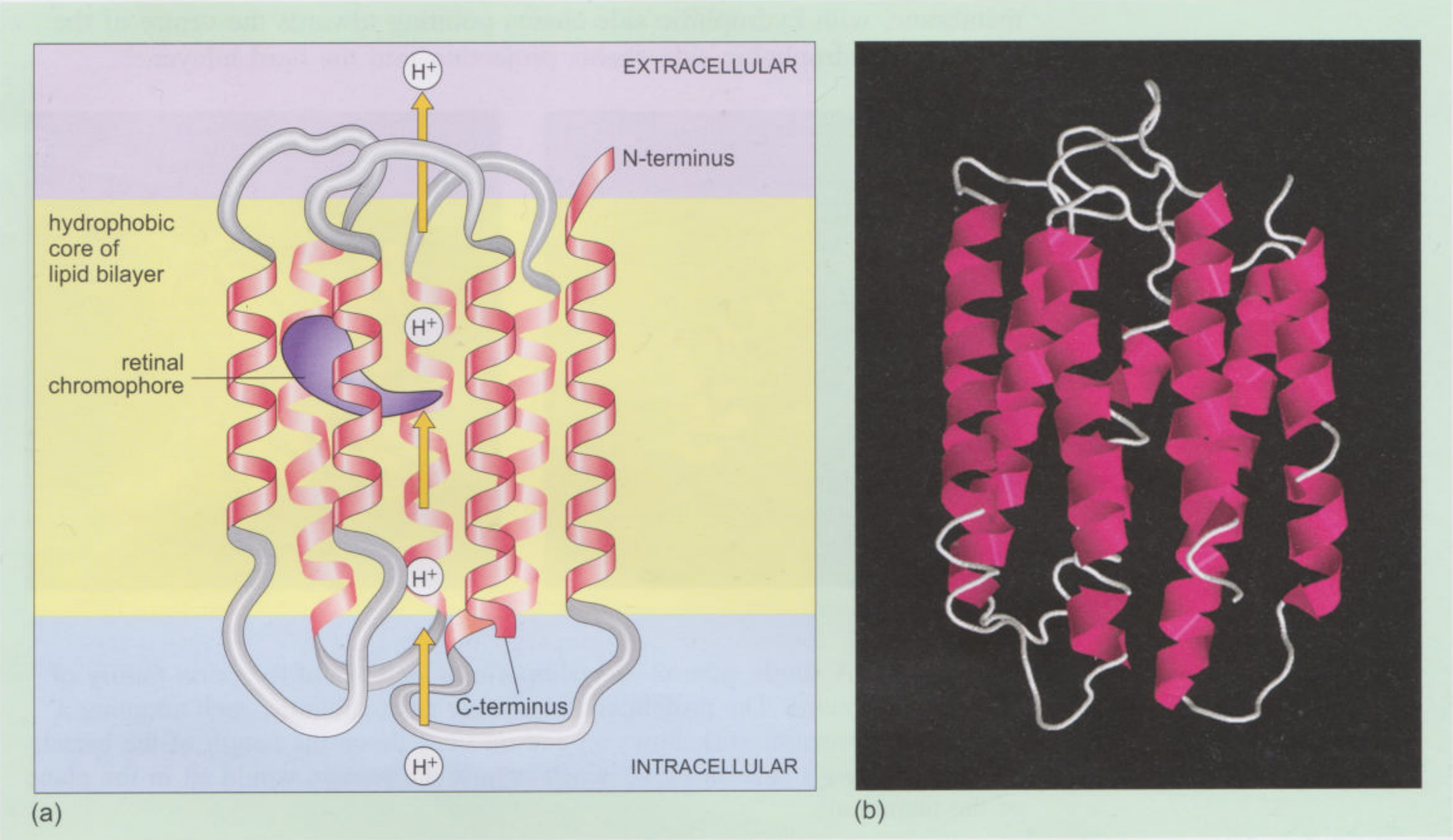


Figure 2.13 (a) Structure of bacteriorhodopsin, a multipass transmembrane protein containing seven α helices linked by extracellular and intracellular stretches of polypeptide. The retinal molecule, represented in purple, is a chromophore (a light-absorbing chemical that gives the protein its purple colour); it functions as a cofactor and is required for the light-activated pumping of protons by bacteriorhodopsin. (b) Three-dimensional representation of bacteriorhodopsin, coloured according to protein secondary structure (α helix, pink; random coil, white).

While glycophorin and bacteriorhodopsin are examples of α helical transmembrane proteins, there are also transmembrane proteins in which the membrane-spanning portion is composed of β sheet in a β barrel conformation (protein 3 in Figure 2.11, Figure 2.14). These β barrel membrane proteins are found in outer membranes of bacteria, mitochondria and chloroplasts. They include porins, a family of pore-forming proteins, where the β barrel forms a channel through which specific hydrophilic molecules can cross the membrane. Maltoporin, for example, is found in Gram-negative bacteria where it provides a route for the diffusion of sugars (such as the disaccharide maltose) from the external environment into the cell.

Many transmembrane proteins, such as the α helical bacteriorhodopsin and the β barrel maltoporin, serve as routes through which hydrophilic solutes can cross the hydrophobic core of the membrane. They do so by creating a hydrophilic environment in their interior. However, the way in which this is achieved is different in these two kinds of transmembrane protein (Figure 2.15). Thus in multipass α helical transmembrane proteins, the helices form bundles with a central channel lined with hydrophilic residues, which are screened off from the hydrophobic membrane. In contrast, in β barrel transmembrane proteins, the β sheet formed by the strands of polypeptide curls around on itself to form a barrel structure opening onto each side of the membrane, with hydrophilic side chains pointing towards the centre of the barrel and hydrophobic side chains projecting into the lipid bilayer.

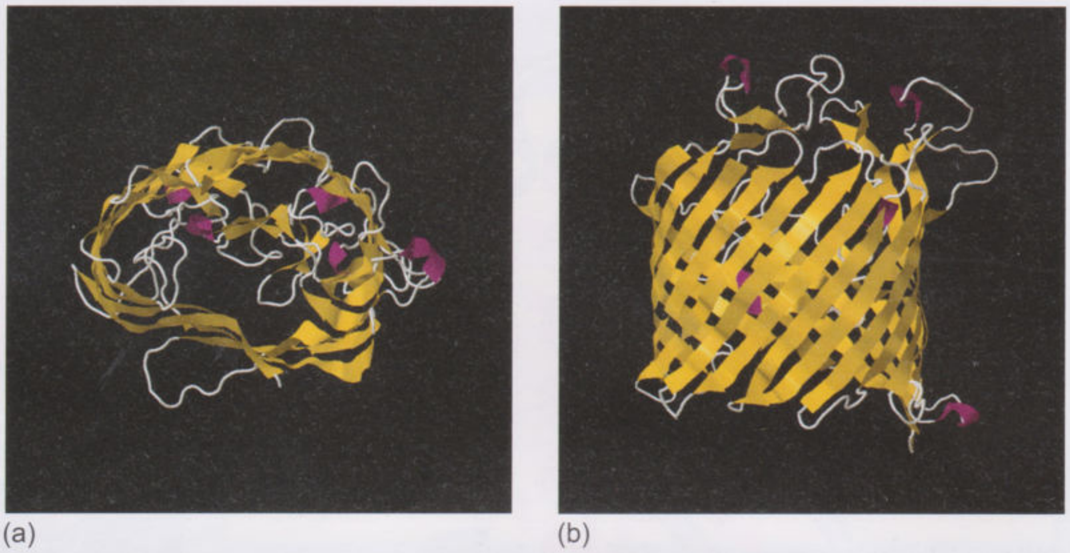


Figure 2.14 A single subunit of maltoporin, a member of the porin family of membrane proteins. The protein contains three such subunits, each adopting a β barrel conformation. (a) shows an end-on view down the length of the barrel, while (b) shows a side-on view, which is how the protein would sit in the plane of the membrane.

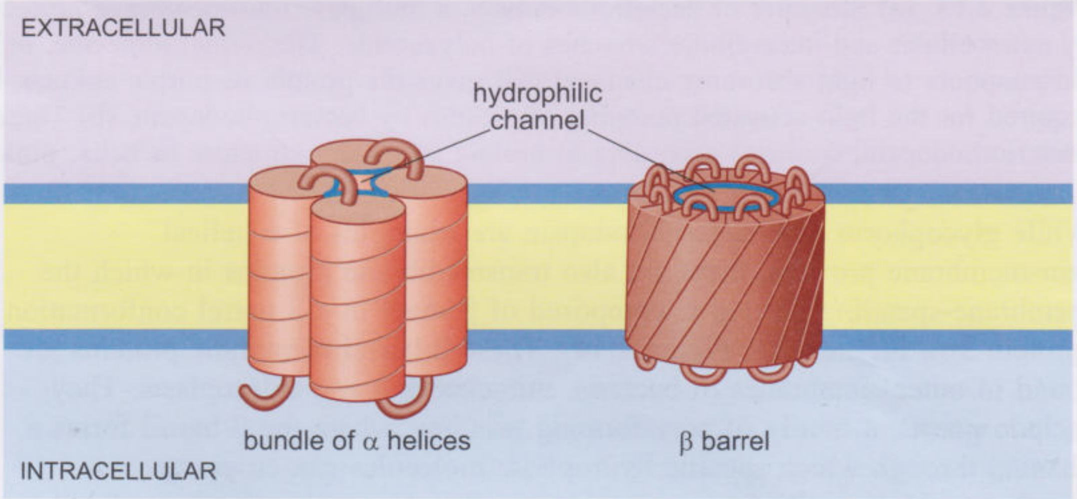


Figure 2.15 Schematic diagram showing how bundles of α helices or a β barrel structure can create a channel across membranes through which hydrophilic solutes can pass or be transported.

Peripheral membrane proteins (proteins 5–7 in Figure 2.11) have no well-defined hydrophobic surface and associate with the membrane via hydrogen bonds and electrostatic interactions with the lipid head groups or other membrane proteins. The proteins that mediate attachment of integral membrane proteins to the cell cytoskeleton are examples of peripheral proteins. In red blood cells, as illustrated in Figure 2.6, the main peripheral proteins are spectrin and the link proteins (ankyrin and band 4.1), which are bound to the inner surface of the cell membrane and form a meshwork that supports and strengthens the membrane, helping the erythrocyte to maintain its shape. The hydrogen bonds and ionic bonds that mediate the association of peripheral proteins with the membrane can be disrupted by high concentrations of salts, a change in pH or, in some cases, the presence of agents that can complex with and sequester calcium ions (Ca^{2+}). Peripheral proteins are therefore relatively easy to dissociate from membranes without the need for detergents or organic solvents to disrupt the lipid bilayer.

Lipid-linked proteins (proteins 8 and 9 in Figure 2.11) are membrane proteins that associate with the lipid bilayer via a covalently linked lipid group, which anchors the protein in the membrane. There are a number of different types of lipid groups in lipid-linked proteins and these differ between lipid-linked proteins that are anchored at the cytosolic surface of the cell membrane and those that are anchored at the extracellular surface. The lipid modification of these proteins is often reversible and this can be a way in which the localisation of the protein and its activity are controlled. Lipid-linked proteins that are anchored at the extracellular surface of the cell membrane include some enzymes and receptors. An example is the enzyme acetylcholinesterase, which catalyses the hydrolysis of the neurotransmitter acetylcholine, thereby terminating its action at nerve terminals. Another interesting example of lipid-linked proteins is those found on the unicellular parasitic protist *Trypanosoma brucei* (which causes sleeping sickness in humans). When challenged by host antibodies against these surface proteins, the parasite can shed the protein by breaking the covalent linkage to the lipid anchor and express a new variant of the protein that is sufficiently different as to escape recognition by the host antibodies.

Summary of Section 2.4

- The three major types of membrane lipid are phospholipids, glycolipids and sterols (cholesterol in animal cells, phytosterols in plant cells and hopanoids in prokaryotes).
- The proportions of particular types of lipid differ in different types of membrane.
- In phospholipids, the hydrophilic head group contains a negatively charged phosphate group, while in glycolipids the head group is a sugar residue.
- Both phospholipids and glycolipids have two long unbranched hydrocarbon chains, which derive from fatty acids or from sphingosine.
- Saturated fatty acyl chains contain only carbon–carbon single bonds; unsaturated fatty acyl chains contain one or more carbon–carbon double bonds.
- The lipid components of membranes can be analysed using a technique known as thin-layer chromatography (TLC).
- Membrane proteins fall into one of three categories. Integral membrane proteins have some portion that interacts with the hydrophobic core of the lipid bilayer; peripheral proteins associate with the membrane indirectly via non-covalent interactions with other membrane proteins or directly via interactions with the hydrophilic head groups of the lipids, but do not interact with the hydrophobic core; lipid-linked membrane proteins are

anchored in the bilayer by means of a lipid linkage at the cytosolic surface or the extracellular surface.

- Transmembrane integral proteins span the lipid bilayer. Depending on the protein, the transmembrane region is either a single α helix, a bundle of α helices, or a β barrel structure.

2.5 Characteristics of biological membranes

2.5.1 Membranes are dynamic structures

Consistent with the fluid-mosaic model, both lipids and proteins can diffuse relatively freely in the plane of the membrane. Lipids and some proteins are also free to rotate around their own long axis. While membrane proteins *never* flip from one membrane leaflet to the other, lipids can 'flip-flop' between the two leaflets; though, when this occurs passively (known as transverse diffusion), it occurs at a much lower frequency than does lateral movement within the plane of the membrane.

■ Why do you think flip-flop of membrane lipids occurs much less often than lateral diffusion?

□ Flip-flop requires the passage of the polar head group of the lipid through the hydrophobic interior of the bilayer, which would be energetically unfavourable.

There are, however, specific enzymes that catalyse the flip-flop of lipids, allowing the efficient remodelling of membranes and the redistribution of the lipid components. This is particularly important when new membrane lipids are synthesised and introduced to established membranes. When newly synthesised membrane from the endoplasmic reticulum fuses with the cell membrane (this is called exocytosis; see Section 2.9 and Book 1, Section 3.4.8), the distribution of phospholipids is disrupted and specific enzymes catalyse flip-flop of lipids to restore and maintain the appropriate distribution of lipids in the cell membrane.

A further important element of lipid mobility is the exchange of lipids between membranes. Like transverse diffusion, exchange of lipids between membranes is slow. Such lipid exchange is made possible by special proteins that can transfer specific phospholipids across the aqueous gap between two adjacent membranes. Cholesterol, having only a weakly polar group, can diffuse passively between the two leaflets of a membrane and also between two juxtaposed membranes.

2.5.2 Membrane stability

Although the interactions between the molecules that make up membranes are almost exclusively weak non-covalent interactions, there are so many of them that membranes are remarkably stable structures in their normal, aqueous environment. The tendency of membrane lipids to remain associated in the typical bilayer array is so strong that fragments of membrane remain after heat, mechanical disruption or chemical attack has disrupted cells, the membrane fragments closing up to form liposomes (Figure 2.3). An impressive illustration of this phenomenon is the greasy appearance of lakes and reservoirs evident after hot, sunny spells, due to the disrupted membranes and resealed membrane fragments of billions of microscopic algal cells, both living and dead.

The maintenance of membrane integrity is critically dependent on temperature. In many animal and plant cells, death from cold is due mainly to irreversible disordering of membranes, caused by formation of ice crystals. Almost all structural proteins and the majority of enzymes are perfectly preserved at temperatures far below 0 °C, but most cells and whole organisms are killed by freezing unless immersed in or perfused with a cryoprotectant, which prevents ice formation. In the absence of cryoprotectant, freezing causes membranes to lose their selective permeability to different kinds of small molecules and ions, so that cells no longer maintain the appropriate intracellular concentrations of these substances. Another type of cold-induced change in membrane structure is the disruption of series of membrane-bound enzymes catalysing consecutive steps in metabolic pathways, so that the enzymes are no longer in the correct alignment to work together.

The main determinant of the temperature range over which a membrane is stable is its lipid composition – in particular, the relative proportions of saturated and unsaturated fatty acids, and the cholesterol content. For example, bananas are native to tropical regions and their cell membranes have a lipid composition that makes them stable at warm temperatures. Ripening involves enzymatic softening of the carbohydrates in the fruit pulp, and also enzyme-catalysed oxidation reactions which cause the familiar blackening of 'over-ripe' bananas. These processes are greatly accelerated if the bananas are cooled to below 10 °C. You learnt in the previous chapter (Section 1.8.3) that enzyme-catalysed reactions are generally slower at lower temperatures; so this hastening of ripening is not dependent on the properties of the ripening enzymes. In fact it arises from the temperature sensitivity of the membranes that normally keep these enzymes separate from their substrates.

2.5.3 Membrane fluidity

As has already been mentioned, the efficient functioning of membrane proteins in transport processes, signalling events, and intercellular communication is critically dependent on the fluidity of the membrane, as interaction with other membrane proteins and intracellular or extracellular ligands depends on this property of the membrane. However, a membrane that is too fluid may also present a problem, making the cell more fragile and vulnerable to physical damage.

Any particular lipid bilayer has a characteristic 'melting' temperature, termed the **transition temperature** (represented as T_m), below which it changes from a fluid state to a solid gel-like state. T_m is directly related to the fluidity of the lipid bilayer: the lower the T_m, the more fluid the bilayer is at any given temperature.

The T_m for a lipid bilayer consisting of a variety of different lipids depends on the T_m of the different lipid components and their relative proportions in the membrane. There are two main ways in which organisms control membrane fluidity: by changing their fatty acid composition or, in the case of animal cell membranes, by changing the cholesterol content.

Unsaturated fatty acids in animal, plant and protist membranes nearly always have C=C double bonds of the kind represented in Figure 2.9a, described as '*cis*'. As this figure shows, a *cis* double bond produces a bend in the chain. The presence of *cis* unsaturated chains, with their bends, prevents very close packing of the fatty acyl chains. This has the consequence of making the membrane more fluid. The T_m of fatty acyl chains decreases with decreasing chain length and increasing unsaturation. Thus membranes with a high proportion of saturated and/or long fatty acyl chains are less fluid than those with a high proportion of unsaturated and/or short fatty acyl chains.

The membrane composition of bacteria living in extremely cold or hot environments is discussed in Book 3, Chapter 2.

Many animals, plants and protists control the fluidity of their cell membranes by varying the relative proportions of saturated and unsaturated membrane fatty acids. *E. coli* provides a good example of how an organism can maintain optimal membrane fluidity in this way. When the environmental temperature drops from 40 °C to 10 °C, the ratio of unsaturated to saturated fatty acids in the cell membrane of this organism increases by more than sevenfold, from 0.4 to 2.9. This change decreases T_m for the membrane and ensures that membrane fluidity is maintained despite the drop in temperature. Plant cells too demonstrate alterations in their membrane fatty acid composition in response to changing environmental temperature. Thus the desert shrub *Atriplex lentiformis* responds to high temperatures by decreasing the level of unsaturated fatty acids and increasing the level of saturated fatty acids in its cell membranes, thereby increasing their T_m.

■ In theory, the fluidity of a lipid bilayer could be increased (i.e. T_m decreased) by reducing the proportion of long-chain saturated fatty acids and increasing the proportion of shorter saturated fatty acids. In practice, however, most organisms do not regulate membrane fluidity in this way. Why might this be?

□ Shorter saturated fatty acids will have a lower T_m; however, the lipid bilayer that they form will be thinner than that formed by longer fatty acids. Thus regulation of membrane fluidity in this way would have the disadvantage of altering the overall membrane structure.

■ What type of phospholipids would you expect to be increased in the cell membrane of arctic fish in winter?

□ The proportion of unsaturated phospholipids in the cell membrane would be increased as a higher proportion of low T_m phospholipids would be required to maintain membrane fluidity at low temperatures.

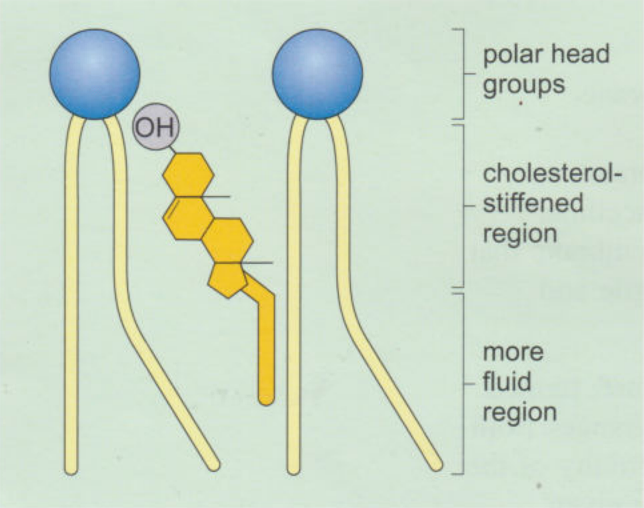


Figure 2.16 Cholesterol packs between phospholipid molecules and restricts movement of fatty acyl chains.

In animal cells, cholesterol is a key regulator of membrane fluidity. The incorporation of cholesterol into phospholipid bilayers is represented in Figure 2.16. The hydrocarbon tail of the cholesterol molecule is flexible, but the four-ring section is very rigid and its hydrophobic interaction with the surrounding fatty acyl tails makes that part of the membrane less deformable and so less fluid. This effect is temperature-dependent as, at lower temperatures, cholesterol helps to disrupt the close packing of fatty acyl chains that occurs in formation of the gel-like state. Thus cholesterol may decrease membrane fluidity at high temperatures and increase it at low temperatures.

2.5.4 The asymmetrical distribution of membrane lipids and proteins

From earlier discussions of membrane proteins, it will be clear that these are distributed asymmetrically in the membrane, with particular proteins being located on one side or the other or, in the case of transmembrane proteins, orientated in a particular way in the lipid bilayer. The distribution of lipids between the two leaflets is also asymmetric.

Thus, in the cell membrane, the cytosolic and extracellular leaflets can differ considerably not only in the type of lipids that are found there (or their relative proportions), but also in the degree of unsaturation of fatty acids in the phospholipid component. The distribution of lipids relates to their particular function. Thus glycolipids (as with glycosylated proteins) are almost exclusively found in the extracellular leaflet of the cell membrane. Here the carbohydrate groups extend into the extracellular environment, where they participate in signalling and recognition events. In contrast, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine, lipids that participate in transmission of signals from the cell membrane to the interior of the cell, predominate in the cytosolic leaflet.

The asymmetric distribution of lipids can be demonstrated experimentally by using enzymes called phospholipases, which hydrolyse phospholipids, removing a single fatty acyl chain. Since phospholipases cannot cross the cell membrane, only those phospholipids that are in the outer leaflet are susceptible when intact cells are treated with the enzyme. Following such treatment, the lipids can be extracted and analysed by TLC (Box 2.2).

Summary of Section 2.5

- Lipids and proteins can diffuse relatively freely in the plane of the membrane; while lipids can flip-flop between the two leaflets of the membrane, proteins never do so.
- The large number of non-covalent interactions between the constituent molecules of membranes makes them very stable.
- The efficient functioning of membrane proteins is critically dependent on the fluidity of the membrane.
- The transition temperature (T_m) of a lipid bilayer is the temperature below which it changes from a fluid state to a solid gel-like state. The lower the T_m, the more fluid the bilayer is at any given temperature.
- Organisms control the fluidity of their membranes by changing the fatty acid composition or, in the case of animal cells, by changing the cholesterol content.

- Membranes with a high proportion of saturated and/or long fatty acyl chains are less fluid than those with a high proportion of unsaturated and/or short fatty acyl chains.
- Cholesterol decreases membrane fluidity at high temperatures and increases it at low temperatures.
- Lipids and proteins are distributed asymmetrically in membranes.

2.6 Refining the fluid-mosaic model: lipid rafts

Singer and Nicolson's fluid-mosaic model of membrane structure proposed a homogenous distribution of proteins, uniformly soluble in the lipid bilayer. This view has been revised in recent years following the development of the **lipid raft hypothesis**. According to this hypothesis, membranes contain microdomains (rafts) which are highly ordered (Figure 2.17). Relative to the rest of the membrane, these rafts are enriched in sphingolipids with saturated fatty acyl chains and cholesterol, and the interactions between these lipids drive formation of the rafts. Their particular lipid composition means that lipid rafts are more cohesive and less fluid that the rest of the membrane.

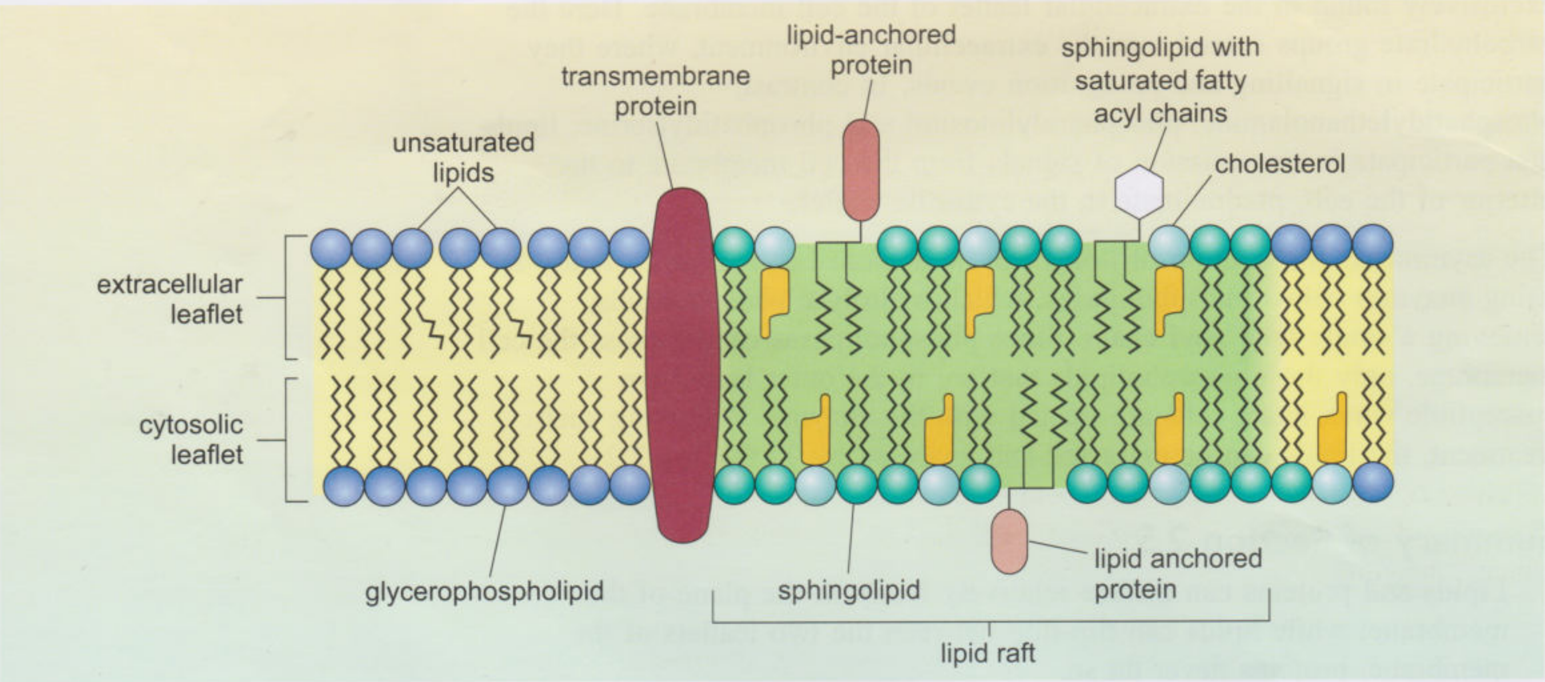


Figure 2.17 Sphingolipid and cholesterol molecules form highly ordered clusters or microdomains, termed lipid rafts, within the fluid membrane. Lipid-anchored membrane proteins that contain saturated lipid groups interact preferentially with these lipids and so concentrate in the lipid rafts, but many proteins are excluded.

Thus lipid rafts can be envisaged as 'islands' in a sea of fluid membrane. Lipid rafts are enriched in lipid-anchored membrane proteins that contain saturated lipid groups and which interact preferentially with the lipids in the raft. In contrast, lipid-anchored membrane proteins that are linked to unsaturated hydrocarbon chains tend to be excluded from lipid rafts. Some transmembrane proteins have also been identified in lipid rafts. Association of proteins with lipid rafts has important consequences for their function. Among those proteins that co-isolate with lipid rafts are many that are involved in cell signalling, suggesting that lipid rafts act as signalling ‘hot-spots’.

■ What advantage might there be in co-localising signalling proteins in this way?

□ Co-localisation of signalling proteins in lipid rafts would facilitate their interactions and allow integration of different signalling processes.

Lipid rafts cannot be identified by conventional light microscopy techniques, because their size is less than the lower limit (300 nm) of detection. A technique known as the fluorescence resonance energy transfer technique (FRET; Box 2.3), in which lipids are ‘tagged’ with fluorescent molecules called fluorophores, has been used to determine the distribution and proximity of lipid-anchored proteins or lipids at the extracellular surface. This experimental approach indicates when two differently tagged proteins or lipids are within 10 nm of each other. From such studies it has been determined that lipid rafts are about 70 nm in diameter and that they are very dynamic structures – proteins constantly move in and out of them and the rafts themselves coalesce and split. A very specific subtype of lipid rafts, known as **caveolae**, form flask-shaped invaginations (inward folds) of the cell membrane, 70–100 nm in diameter (Figure 2.18). Caveolae have been implicated in endocytosis, a topic that is discussed in Section 2.9.2.

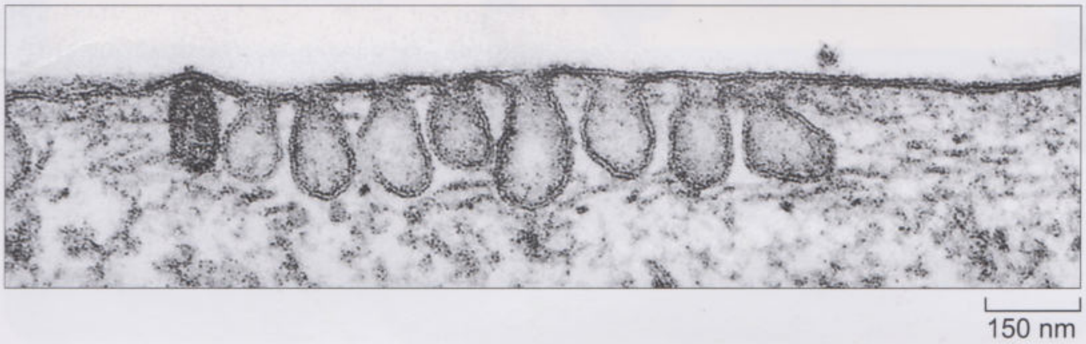


Figure 2.18 Caveolae in the cell membrane of a fibroblast. Caveolae are a special type of lipid raft distinguished by their flask-like structure and the presence of the protein caveolin.

Box 2.3 The fluorescence resonance energy transfer technique (FRET)

FRET is used to determine the proximity of two molecules on a cell surface. The two molecules are labelled with different fluorophores. Labelling can be achieved directly or indirectly, using fluorescently labelled antibodies (as depicted in Figure 2.19). One of the fluorescent tags is termed the donor and the other is termed the acceptor and these are chosen according to their excitation and emission wavelengths. In Figure 2.19, protein A has been labelled using an antibody conjugated to the fluorophore fluorescein (the donor) and protein B has been labelled with an antibody conjugated to a different fluorescent molecule, rhodamine (the acceptor). The cells are illuminated with UV light at a wavelength that excites the donor fluorophore (but not the acceptor fluorophore), causing it to emit fluorescent light at a particular wavelength. If proteins A and B are sufficiently close to each other (<10 nm), energy from the donor emission is transferred to the acceptor, causing it to emit light at a wavelength characteristic of the acceptor. In the case of rhodamine, the acceptor in this example, the emission is visible red light, which can be detected using a fluorescence microscope.

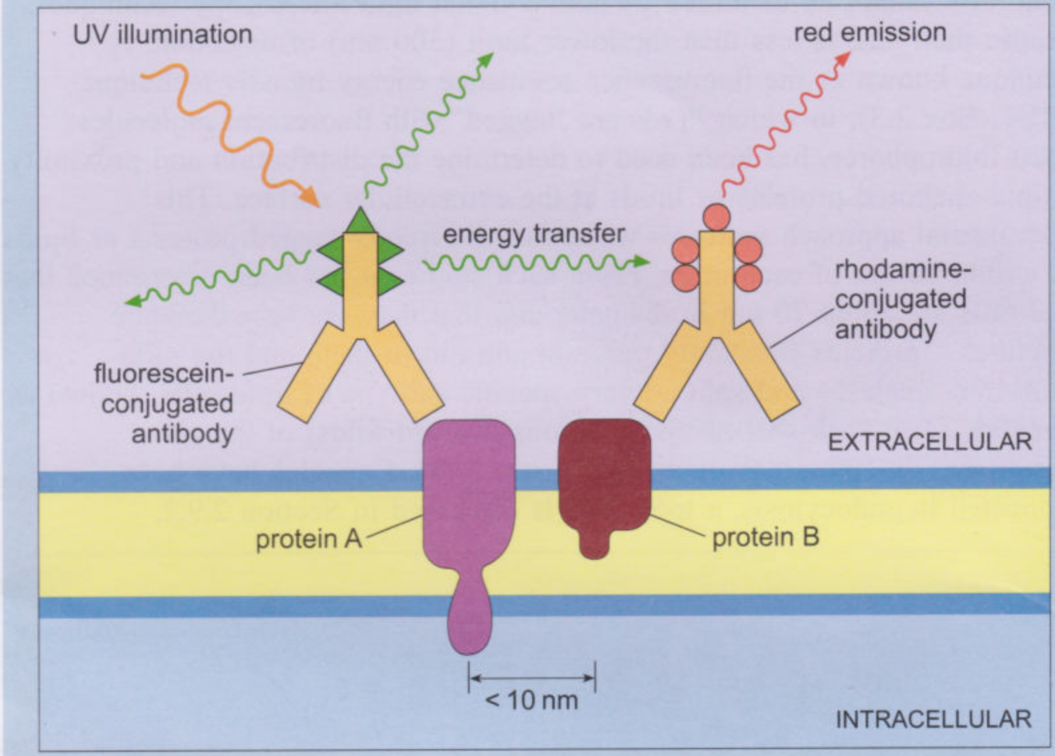


Figure 2.19 The fluorescence resonance energy transfer technique (FRET).

Summary of Section 2.6

- Lipid rafts are highly ordered microdomains that float in the membrane. They are enriched in cholesterol and lipids containing saturated fatty acyl chains, a composition that makes them more cohesive and less fluid than the rest of the membrane.
- Among those proteins that co-isolate with lipid rafts are many that are involved in cell signalling, suggesting that lipid rafts act as 'hot-spots' for intercellular signalling.
- Studies using the fluorescence resonance energy transfer technique (FRET) have shown that lipid rafts are about 70 nm in diameter and are very dynamic structures.
- Caveolae are a type of lipid raft that form flask-shaped invaginations of the cell membrane and have been implicated in endocytosis.

2.7 Passive diffusion of small molecules and ions across membranes

As has already been discussed, membranes demonstrate selective permeability to different solutes; they are permeable to some and impermeable to others.

Diffusion is the spontaneous movement of a solute such that it tends to distribute evenly in the space available to it. A substance will diffuse from where it is most concentrated to where it is less concentrated, i.e. down its concentration gradient, and no energy input is required for this to happen. Where a solute carries a net charge, as is the case for ions, differences in charge also affect diffusion. The combination of the effect of concentration and charge differences acting on an ion is known as an *electrochemical gradient* and is described in more detail in Section 2.8.3.

The two factors that determine how permeable the lipid bilayer is to a solute are firstly its polarity (whether it is non-polar or polar), and secondly its size (Figure 2.20). Thus the lipid bilayer is most permeable to small non-polar molecules such as the gases oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2). At the other extreme, it has very low permeability to inorganic ions. Between these two extremes, for diffusion of uncharged but polar molecules, size is an important factor; with small molecules such as water crossing more readily than larger molecules such as glucose and amino acids.

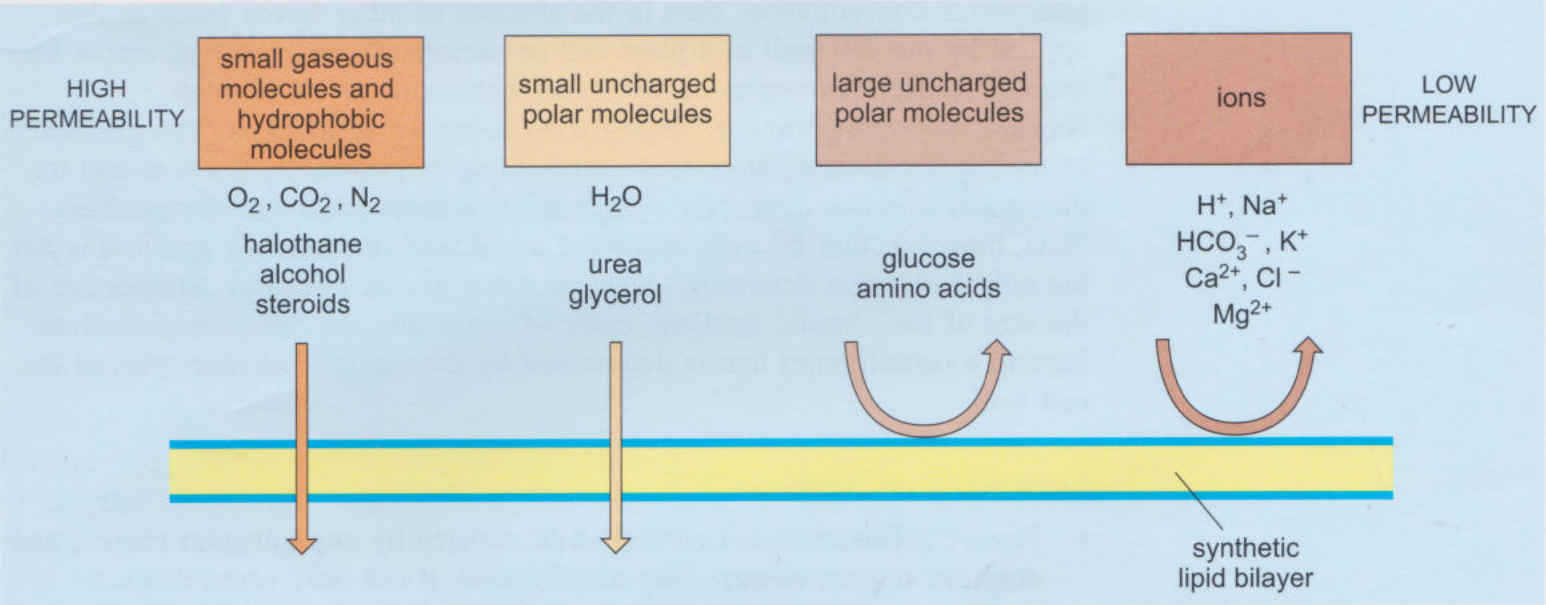


Figure 2.20 The relative permeability of a synthetic lipid bilayer (i.e. one devoid of transport proteins found in natural cell membranes) to different types of molecules and ions. The straight arrows that cross the membrane denote relatively high permeabilities; the curved arrows denote very low permeabilities.

This kind of movement is known as **passive diffusion** and is the most straightforward way in which a solute crosses a membrane. It is not controlled or assisted by any transport protein and in this respect differs from facilitated diffusion (described in Section 2.8.2). Passive diffusion, like all diffusion, can only occur down a concentration gradient (in the case of non-polar solutes) or down an electrochemical gradient (in the case of ions). It does not require energy, and proceeds spontaneously until an equilibrium is reached in which the concentration of the solute is the same on both sides of the membrane.

2.7.1 Osmosis – diffusion of water across membranes

In considering movement of molecules into and out of cells, water deserves particular mention because it is the solvent in which the chemical reactions necessary for life occur and, as such, it is essential to life. Furthermore, unlike other molecules, the passive diffusion of water across membranes is not simply a matter of movement down a concentration gradient.

The recovery of a wilted plant after watering and the bursting of red blood cells when placed in water containing few or no ions, are both caused by transmembrane water movement into cells. In plant cells, the presence of a rigid cell wall sets a limit on water uptake and the cells become stiff, or turgid. The same limitation applies to most bacterial cells and fungi, which also have a cell wall. However, in the case of animal cells, bounded only by a relatively delicate cell membrane, water continues to enter, until the outward pressure on the cell membrane causes it to rupture.

What causes movement of water into, or out of, cells? The driving force in the above examples is the difference between the *total* solute concentrations, known as **osmolarity** (moles per litre of solution), on either side of the membrane. The nature of the solutes doesn't matter. If there is a gradient in total solute concentration, then in the absence of other forces (such as that applied by the cell wall in a plant cell or bacterium), water moves across the membrane from the region of low solute concentration to the high concentration compartment. This process is known as **osmosis**. The gradient of total solute concentration thus corresponds to an osmotic gradient and the movement of water across the membrane is a consequence of this gradient. Note, however, that for cells that have a cell wall, the osmotic gradient is not the only factor that determines whether water moves in or out. Irrespective of the size of the osmotic gradient, entry of water into the cell cannot continue beyond a certain point that is determined by the mechanical properties of the cell wall.

Summary of Section 2.7

- Passive diffusion is not controlled or assisted by any transport protein and does not require energy. Like all diffusion, it can only occur down a concentration gradient (in the case of non-polar solutes) or down an electrochemical gradient (in the case of ions).
- How readily a solute will diffuse across a lipid bilayer depends on its polarity and its size.
- The passive diffusion of water across membranes is driven by osmosis. For plants, fungi and bacteria, which have cell walls, entry of water is limited by the mechanical properties of the cell wall.

2.8 Transport mediated by membrane proteins

The permeability of artificial lipid bilayers to solutes (such as is represented in Figure 2.20) is markedly different to that of real biological membranes. Early observations to this effect contributed much to our current understanding of membrane structure and in particular the importance of membrane proteins. The permeability of membranes to water, for example, used to be ascribed to passive diffusion alone, dictated by the osmotic gradient. However, the rate of movement of water across the membranes of red blood cells is more than twice as large as could be accounted for by diffusion, and is inhibited by protein-binding chemicals.

■ What does this evidence suggest?

□ That there are transport proteins that facilitate movement of water across membranes.

Indeed, it was subsequently found that there is a family of integral membrane proteins called aquaporins, which form highly selective channels through which water can cross the membrane. Slightly different aquaporins are found in animals, plants, fungi and bacteria. The importance of aquaporins is demonstrated in several diseases of humans in which the aquaporin of a particular tissue is functionally defective: for example, in the mammalian kidney, where a reduced ability to reabsorb water from the collecting ducts (after filtration of the blood) results in the production of copious amounts of watery urine, a condition called diabetes insipidus. Water transport is also a major activity of the gut: fluid is secreted from salivary glands, stomach and small intestine and much of it is absorbed again from the colon. Several aquaporins are involved in this water transport: for example, AQP4 in colonic surface epithelium facilitates water uptake from the colon into the bloodstream. In plants, aquaporin channel activity has been demonstrated in the membrane that surrounds the vacuole.

The aquaporins are just one type of transport protein. Transport proteins are integral membrane proteins that transport small molecules (particularly metabolites) and ions across membranes and, depending on their mode of operation, they are known as carriers or channels.

2.8.1 Carriers and channels

The 'policing' of molecular and ionic traffic across the cell membrane (and across internal membranes too) is essential to the integrity of every cell, and the relative abundance of the different types of membrane transport proteins confers unique properties on particular tissues or groups of cells within a single tissue.

The membrane proteins that determine what substances enter and leave the cell are of two main types, classified on the basis of the mechanism by which the material is transferred across the membrane: **carriers** (or transporters) and **channels** (Figure 2.21). Carriers and channels are multipass transmembrane proteins that present a hydrophobic surface towards the membrane's lipid interior and a hydrophilic surface towards the aqueous environment. Both types solve the problem of lipid impermeability to polar substances, either by masking the polarity of the molecule or ion to be transported, or by neutralising the non-polarity of the membrane interior (by forming a polar channel through it).

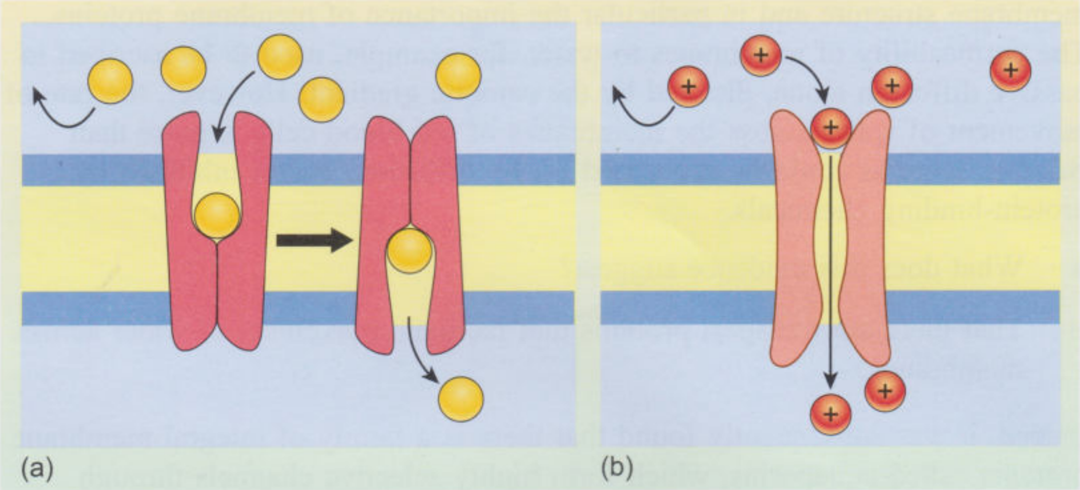


Figure 2.21 Model of membrane transport by (a) carrier proteins and (b) protein channels. The transferred substances are shown as coloured balls; note that in the case of protein channels, the substance that is transferred is usually an ion, as represented here. The bold arrow in (a) denotes a conformational change in the carrier protein which causes the solute binding site to shift from one side of the membrane to the other.

A carrier, as the name implies, binds to a solute molecule (or ion) on one side of the membrane and releases it on the other side. For example, the glucose carrier protein in mammalian cell membranes binds glucose present in the extracellular fluid, where it is at a higher concentration than inside the cell, and releases it into the cytosol for use in metabolism. The carrier is specific for glucose; that is, it does not bind other sugars (or binds them much less strongly). The binding of the solute molecule to be transported alters the conformation of the carrier protein in such a way that the binding site, occupied by the solute molecule, becomes exposed on the opposite side of the membrane, so the carrier can then release its 'cargo' into the right compartment (Figure 2.21a).

Some carrier proteins, such as the glucose transporter in red blood cell membranes, transport only one type of solute across the membrane; such transport is known as **uniport**. Other carriers that transport two different solutes at a time are known as **cotransporters** and, depending on whether the two solutes are transported in the same or in opposite directions, this transport is known as, respectively, **symport** or **antiport** (Figure 2.22). (Note that the terms uniport, symport and antiport are used to describe both the type of transport and the carrier protein itself.) For many cotransporters, one of the two solutes that are transported is an ion. (You will encounter some examples of cotransporters in subsequent sections.)

The other type of membrane proteins that transfer hydrophilic substances (in this case usually ions) across membranes are channels (Figure 2.21b). These

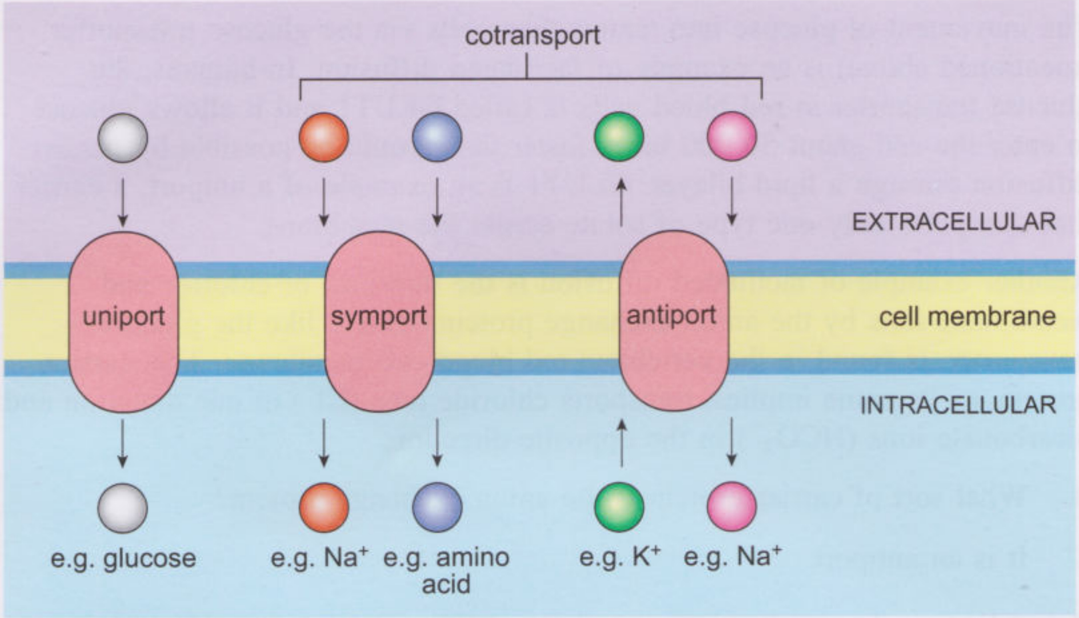


Figure 2.22 Types of carrier-mediated transport. Where only one solute is transported, this is known as uniport; cotransport entails transport of two different solutes at the same time, either in the same direction (symport) or in opposite directions (antiport).

proteins generally consist of between four and six subunits, assembled to form a pore in the membrane through which solutes can pass. Like carriers, channels are very specific, i.e. only selected ions or molecules can pass through. Their selectivity is determined by the amino acid side chains that line the pore and the diameter of the narrowest part of the pore. The feature that most clearly distinguishes the operation of a carrier from a channel is the rate of solute transfer; transfer through ion channels is at least 100 times faster than transfer by ion carriers.

Many ion channels open and close in response to a particular stimulus and are involved in communication between cells. For some of these so-called *gated* ion channels, the stimulus is electrical, for example the voltage-gated potassium channels of nerve cells (Section 2.8.4); for others, binding of a specific signalling molecule causes the channel to open or close. (The role of ion channels in intercellular signalling is discussed in some detail in Chapter 4.)

2.8.2 Facilitated diffusion

Facilitated diffusion is the diffusion of a solute across a membrane via either a carrier or channel protein. As for passive diffusion, no energy is required for this to occur because the direction of movement is down the concentration gradient (or, in the case of ions, down the electrochemical gradient). The carrier or channel is merely providing a path through the hydrophobic interior of the lipid bilayer, facilitating the 'downhill' diffusion of the solute. The fact that energy is not required for facilitated diffusion via channels and carriers does not mean that movement of solutes in this way is uninhibited, and many channels and carriers are controlled (gated) by means of electrical or chemical signals.

The movement of glucose into mammalian cells via the glucose transporter (mentioned above) is an example of facilitated diffusion. In humans, the glucose transporter in red blood cells is called GLUT1 and it allows glucose to enter the cell about 50 000 times faster than would be possible by passive diffusion through a lipid bilayer. GLUT1 is an example of a uniport, a carrier that transports only one type of solute across the membrane.

Another example of facilitated diffusion is the transport of chloride and bicarbonate ions by the anion exchange protein, which, like the glucose transporter, is found in the vertebrate red blood cell membrane. This carrier protein, as its name implies, transports chloride ions (Cl^-) in one direction and bicarbonate ions (HCO_3^-) in the opposite direction.

■ What sort of carrier protein is the anion exchange protein?

□ It is an antiport.

Depending on the relative concentrations of Cl^- and HCO_3^- inside and outside the red blood cell, the anion exchange protein can operate in either direction. This is an important mechanism for the removal of carbon dioxide (CO_2) from tissues and its delivery to the lung. Like other carrier proteins, the anion exchange protein is thought to function by switching between two conformational states. However, it requires both solutes to bind at the same time on opposite sides of the membrane before the conformational change and the resulting exchange of anions can occur.

2.8.3 Ion transport and membrane potential

Intracellular ion concentrations differ greatly from those in the extracellular environment. In virtually all cells, including bacterial, plant and animal cells, the cytosolic concentration of K^+ is much higher than that of Na^+. In addition, in both invertebrates and vertebrates, the concentration of K^+ is higher inside cells than outside, while the concentration of Na^+ is lower inside cells than outside, as illustrated for a typical mammalian cell in Table 2.3.

Table 2.3 Concentrations of ions inside and outside a typical mammalian cell.

Ion	Intracellular concentration (mmol l^{-1})	Extracellular concentration (mmol l^{-1})
Na^+	12	145
K^+	139	4
Cl^-	4	116
X^{-*}	138	9

* X^- represents macromolecules that are negatively charged under physiological conditions.

Collectively, the activities of ion channels and ion pumps maintain the ion concentration gradients across the cell membrane. However, there is a difference in net charge across the membrane, with the inside of the cell being negative with respect to the outside. Because of the difference in net charge, there is a difference in voltage (an electrical potential gradient) across the cell membrane. The magnitude of this voltage difference, termed the **membrane potential**, ranges from −10 to −100 millivolts (mV), with a value of −70 mV being typical of many mammalian cell types. Note that, by convention, extracellular voltage is defined as zero and, since the inside of the cell is negative with respect to the outside, the membrane potential has a negative value.

If ions could diffuse freely across membranes, then they would reach an equal concentration on each side (i.e. an equilibrium) and there would be no differences in charge across the membrane. However, while ion channels allow K^+ to enter the cell, the diffusion of Na^+ into the cell is blocked. In addition, a membrane carrier known as the sodium pump actively pumps Na^+ ions out of the cell and K^+ ions in, as will be described in detail in Section 2.8.4.

The potassium ion (K^+) concentration difference contributes most to the cell membrane potential. The proportion of ions involved is tiny, however. For example, it has been calculated that in a typical animal cell of 20 μm diameter, which contains around 6×10^{11} K^+ ions, the movement of only a few million K^+ ions out of the cell would carry enough charge to change the membrane potential by about 100 mV. Thus even small changes in ion concentrations can have very significant effects on membrane potential.

Membrane potential has an important effect on passive and facilitated diffusion of ions. Since the inside of the cell is negative, the membrane potential enhances the entry of positive ions and impedes the entry of negative ions. Thus, for example, for chloride ions (Cl^-) to be transported into a cell by facilitated diffusion, the concentration gradient (high $[Cl^-]$ outside, low $[Cl^-]$ inside) must be large enough to overcome the opposing electrical gradient. The tendency of a particular ion to diffuse across the membrane thus depends not only on its concentration gradient but also on the existing membrane potential. The combination of these forces acting on an ion is called the **electrochemical gradient** for that ion. An electrochemical gradient of a particular ion is an important way in which the cell can store energy. This energy can be used to drive the transport of a solute up a gradient of concentration or charge (i.e. active transport, Section 2.8.5), or to make ATP, as you will learn in the following chapter.

2.8.4 Opening and closing ion channels generates action potentials in nerve cells

In nerve cells, muscle cells and some other cell types, which have the property of being electrically excitable, particular stimuli can trigger a very rapid change in membrane potential. Signalling molecules that reach the neuron surface bind to receptors that, when activated, result in opening or closing of ion channels, depolarising the neuron (causing its membrane potential to become less negative). When the membrane potential of the neuron becomes less negative by about 7–15 mV, voltage-gated sodium channels open. The membrane potential at which voltage-gated sodium channels open to generate a nerve impulse is termed the action potential firing threshold.

- ■ What happens to the membrane potential when the voltage-gated sodium channels are open, and why?
- □ The membrane potential decreases; that is, the inside of the cell becomes more positive, because Na^+ ions move into the cell down their electrochemical gradient.

The rapid and sudden influx of Na^+ ions produces a transient reversal of the membrane potential which is known as the action potential. The inside of the membrane thus becomes more positive than the outside, by about +30 mV. The action potential is propagated to an adjacent region of the neuronal membrane and in this manner it travels down the axon as a wave of depolarisation.

The duration of the action potential in vertebrates (and also flying insects) is very short (milliseconds), but in many invertebrates it can last much longer (up to one second). Shortly after the initial opening of the Na^+ channels, two events occur which return the neuron to its resting membrane potential (i.e. they repolarise the neuron). The first is the opening of voltage-gated K^+ channels, which increases the exit of K^+ ions from the neuron (down their electrochemical gradient), removing positive charge from the intracellular side. The second is the closure of the voltage-gated Na^+ channels, as the membrane potential returns to the resting potential, preventing further net entry of Na^+ ions into the cell.

2.8.5 Active transport

As you have seen, carriers and channels facilitate the transmembrane movement of solutes down an electrochemical or concentration gradient, by a process that does not require an input of energy. However, cells also need to transport certain solutes across their membranes 'uphill'; in other words, *against* an electrochemical or concentration gradient. This process is called **active transport** and requires an energy source. Active transport performs three important functions in cells:

1. It allows essential nutrients to be taken up into the cell even when their intracellular concentration is higher than their extracellular concentration.
2. It allows waste products to be secreted by the cell even when their extracellular concentration is higher than their intracellular concentration.
3. It enables the cell to maintain stable non-equilibrium intracellular concentrations of particular inorganic ions (Na^+, K^+, Ca^{2+} and H^+) and hence preserves the membrane potential.

The membrane proteins that are involved in active transport are carriers and they not only move their specific solute, they also provide a mechanism for coupling movement of the solute to an energy-yielding reaction. The most common source of energy for active transport is hydrolysis of ATP, in which case it is referred to as *primary* active transport. However, energy stored in electrochemical gradients, or light energy, can also drive active transport (known as secondary active transport). Bacteriorhodopsin, a protein that you met in Section 2.4.2, and which is found in the cell membrane of the archaeon

Halobacterium halobium, is an example of a transport protein that uses light energy to pump a solute; protons (H^+) are pumped out of the cell in this case. The resulting proton gradient is used to drive the synthesis of ATP.

As described in Section 2.8.2 (Table 2.3), the concentration of potassium ions (K^+) is much higher inside the cell than outside, while the opposite is true for sodium ions (Na^+).

■ In which direction must Na^+ ions and K^+ ions be actively transported to maintain the concentration gradients for these ions shown in Table 2.3?

□ Na^+ ions must be pumped out and K^+ ions pumped in.

In fact, both these ionic gradients are maintained by the activity of the Na^+–K^+ ATPase or **sodium pump**, a membrane carrier protein that uses the energy of ATP hydrolysis to pump out Na^+ ions and at the same time pump in K^+ ions. The evidence that ATP hydrolysis provides the energy to drive the pump has come from studies on resealed red cell membranes ('ghosts', Section 2.3.3) in which the cytoplasm was replaced by solutions with known concentrations of ions and ATP, and the effects on the operation of the pump recorded. A simple schematic model of the mechanism of the sodium pump is shown in Figure 2.23.

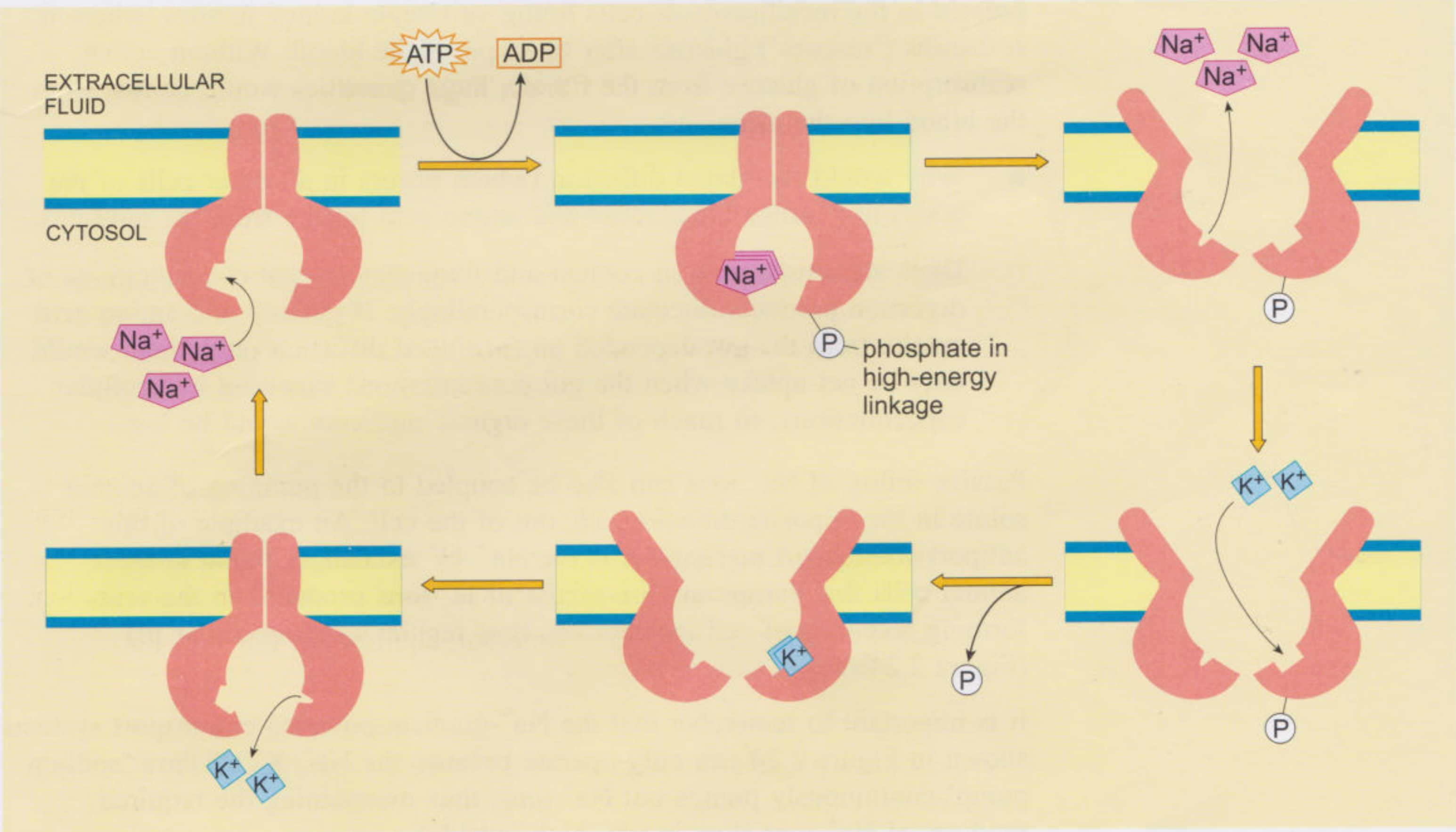


Figure 2.23 A scheme showing the sodium pump, which extrudes three Na^+ ions for every two K^+ ions taken up. Hydrolysis of ATP supplies the energy required for the transport of Na^+ and K^+ against an electrochemical gradient. The protein is phosphorylated by ATP in the presence of Na^+ and dephosphorylated in the presence of K^+.

As you can see, for every molecule of ATP hydrolysed, three Na^+ ions are pumped out of the cell and two K^+ ions are pumped in. The sodium pump is present in virtually all animal cell membranes and consumes up to 30% of the cell's ATP (up to 70% in the case of nerve cells) to maintain the required cytosolic Na^+ and K^+ concentrations.

Animal cells have other ATP-driven pumps that function in intracellular membranes: for example, the Ca^{2+} pump in ER membranes and the proton (H^+) pump of lysosomal membranes required for acidification of the lysosome's interior. Plant cells don't have a sodium pump but have both a Ca^{2+} pump and a proton pump in the plasmalemma (cell membrane).

The sodium pump produces a steep gradient of Na^+ ions across the cell membrane. The dissipation of this gradient, i.e. the flow of Na^+ ions back down their electrochemical gradient, may be coupled to the simultaneous, 'uphill' transport of another solute. This is an example of cotransport (Figure 2.22). Note that the building up of the ion gradient in the first place requires ATP, but the dissipation of the ion gradient thus generated provides the energy for the transport of another solute against its concentration gradient. Cotransport of solutes in the same direction, symport, is the mechanism by which glucose and amino acids are taken up by the epithelial cells lining the gut (see Figure 2.24a). An Na^+-powered glucose transporter is present in the membranes of cells lining vertebrate kidney tubules, where it reabsorbs ('rescues') glucose after filtration of the blood. Without active reabsorption of glucose from the filtrate, huge quantities would be lost from the blood into the urine.

■ Why would facilitated diffusion (which occurs in all other cells of the body) not suffice for glucose and amino acid uptake from the gut?

□ Diets and meals vary in content and frequency, so gut concentrations of digestion products fluctuate correspondingly. If glucose and amino acid uptake from the gut depended on facilitated diffusion only, there would only be net uptake when the gut concentrations exceeded intracellular concentrations, so much of these organic nutrients would be lost.

Passive influx of Na^+ ions can also be coupled to the pumping of another solute in the opposite direction, i.e. out of the cell. An example of this antiport cotransport mechanism is the Na^+–H^+ exchanger found in most animal cells that pumps out the excess of H^+ ions produced in the acid-forming reactions of cell metabolism, thus regulating intracellular pH (Figure 2.24b).

It is important to remember that the Na^+-gradient-powered cotransport systems shown in Figure 2.24 can only operate because the Na^+–K^+ ATPase 'sodium pump' continuously pumps out Na^+ ions, thus maintaining the required gradient of Na^+ ions (low inside, high outside).

Both bacterial and plant cell membranes have an H^+ ATPase which generates a proton gradient across the membrane. In plant root cells, dissipation of this proton gradient is coupled to the uptake of mineral ions from the soil against their concentration gradient, and, in some bacteria, the proton gradient drives flagellar cell movement (Chapter 5).

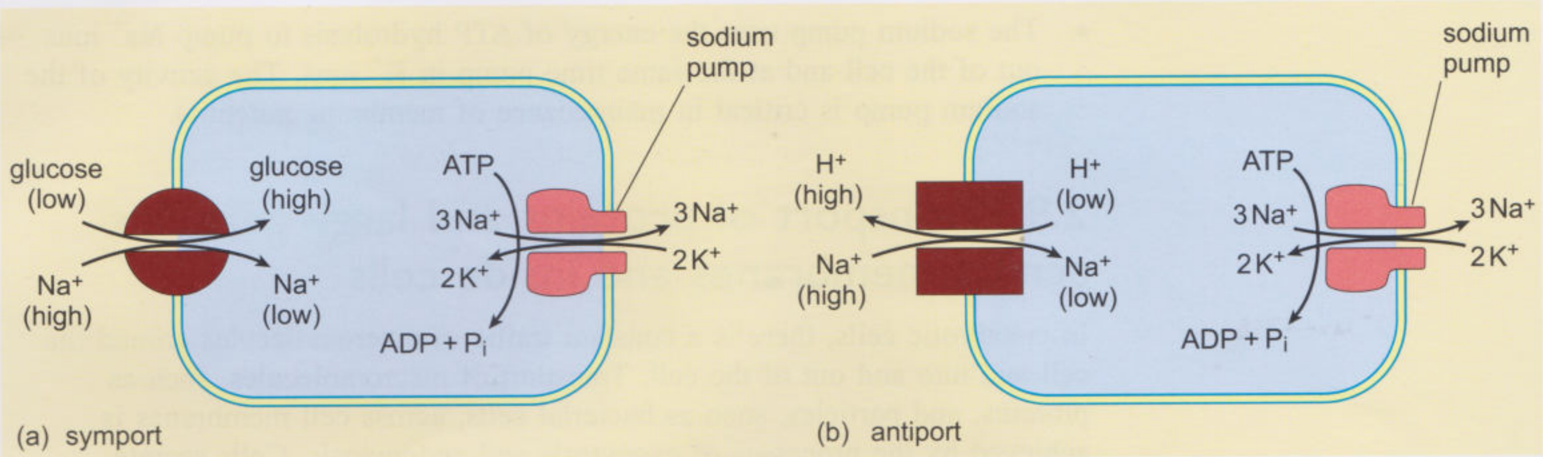


Figure 2.24 (a) Symport: the Na^+–glucose cotransport system. (b) Antiport: the Na^+–H^+ cotransport system.

Summary of Section 2.8

- Binding a solute molecule alters the conformation of a carrier protein such that the solute molecule is transferred. Uniport carriers transport only one type of solute across the membrane (e.g. the glucose transporter on red blood cell membrane). Cotransporters transport two different solutes at a time. The two solutes may be transported in the same direction (symport) or opposite directions (antiport).
- Channels form a pore in the membrane through which solutes can pass. Many ion channels open and close in response to a particular stimulus (electrical or chemical) and are involved in communication between cells.
- Facilitated diffusion occurs via either a carrier or channel protein. No energy is required because the direction of movement is down the concentration gradient (or, in the case of ions, down the electrochemical gradient).
- All cells have a voltage difference across their cell membrane (termed the membrane potential) that results from unequal distribution of ions (and their net charge) on the two sides. The inside of the cell is negative with respect to the outside. Ion concentration gradients are maintained by the activities of ion channels and ion pumps.
- The tendency of a particular ion to diffuse across a membrane depends on both its concentration gradient and the existing membrane potential. The combination of these forces acting on an ion is called the electrochemical gradient for that ion. An electrochemical gradient of a particular ion is an important way in which the cell can store energy.
- In electrically excitable cells such as nerve cells, particular stimuli can trigger very rapid changes in membrane potential, known as an action potential.
- Active transport, the transport of solutes across membranes against an electrochemical or concentration gradient, requires an energy source. In primary active transport, the energy comes from hydrolysis of ATP, while secondary active transport is driven by the energy stored in electrochemical gradients or by light energy.

- The sodium pump uses the energy of ATP hydrolysis to pump Na^+ ions out of the cell and at the same time pump in K^+ ions. The activity of the sodium pump is critical in maintenance of membrane potential.

2.9 Transport of proteins and large particles across membranes and inside cells

In eukaryotic cells, there is a constant traffic of macromolecules around the cell and into and out of the cell. Transport of macromolecules, such as proteins, and particles, such as bacterial cells, across cell membranes is achieved by the processes of exocytosis and endocytosis. Cells secrete substances by exocytosis and take in macromolecules by endocytosis.

Substances are transported within the cell in membrane-bound vesicles. The membrane-bound vesicles can fuse with the membranes of other intracellular compartments (or, in the case of exocytosis, with the cell membrane) allowing transfer of the contents. Figure 2.25 summarises the membrane trafficking routes within the cell and the intracellular compartments involved are represented in Figure 2.26. As you can see, there is transfer in both directions, both out to and inwards from the cell membrane, as well as internal two-way traffic.

Substances produced by the cell that are destined for secretion are delivered to the cell membrane via the secretory pathway (Figures 2.25 and 2.26). In a process similar to the transfer of newly synthesised proteins from the ER (Book 1, Section 3.4.5), vesicles containing substances destined for secretion travel through the internal membrane system, via the Golgi apparatus.

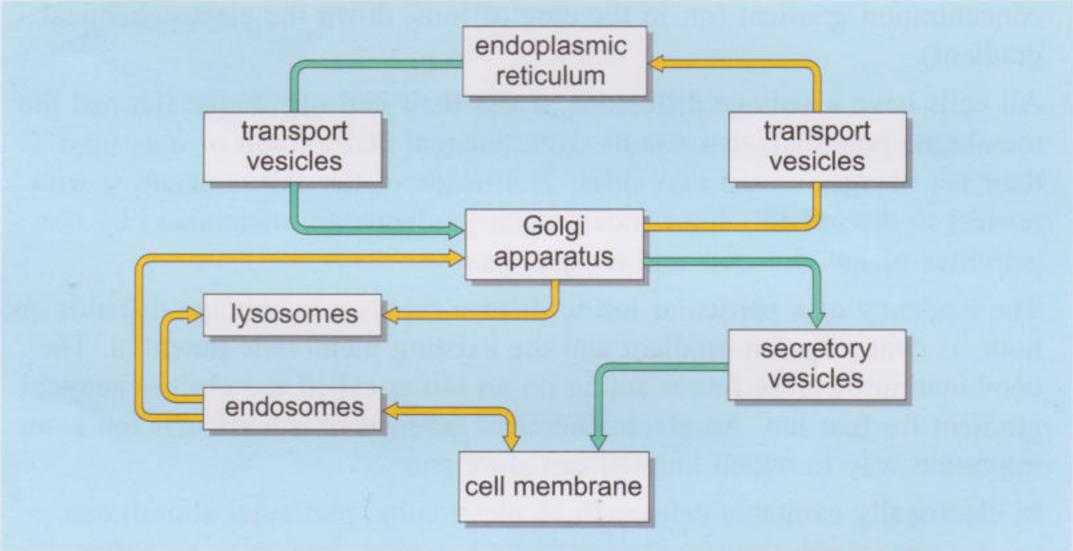


Figure 2.25 Intracellular membrane trafficking 'map' showing the routes by which substances move between intracellular compartments and the cell membrane. The most direct route for substances destined for secretion at the cell membrane (the secretory pathway) is represented by the green arrows.

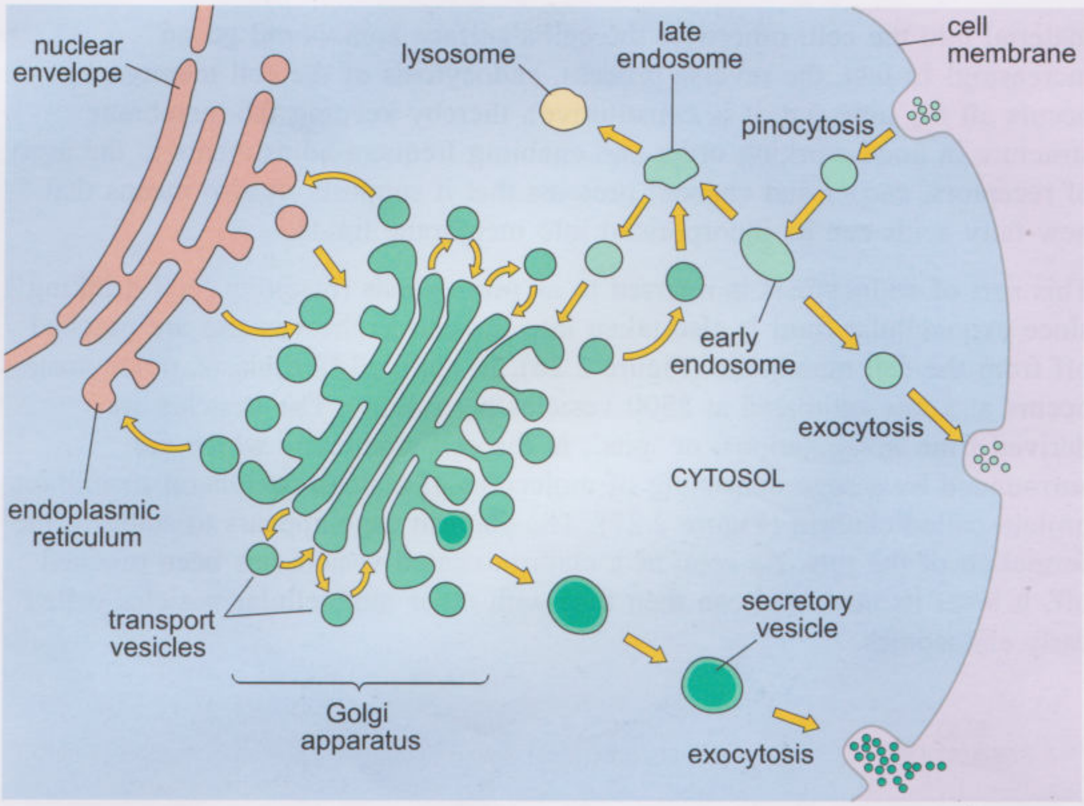


Figure 2.26 Trafficking through the internal membrane system showing the various intracellular compartments and the movements of vesicles between these.

2.9.1 Exocytosis

Exocytosis is the delivery of vesicles to the cell membrane where fusion occurs and the contents of the vesicle are released outside the cell. It can be either constitutive (i.e. occurring all the time) or regulated (i.e. only triggered as required by regulatory processes) (Book 1, Section 3.4.8). In eukaryotic cells, constitutive exocytosis is responsible for the delivery of newly synthesised membrane lipids and proteins in ER-derived vesicles from the Golgi to the cell membrane, and the secretion of certain newly synthesised proteins.

Some proteins are stored in secretory vesicles in the cytosol and are exported by exocytosis only when the process is triggered by a particular extracellular signal. Examples of this regulated exocytosis are secretion of digestive enzymes by cells in the gut, or secretion of hormones or neurotransmitters.

■ What physical property of membranes do you think is particularly important for exocytosis?

☐ During exocytosis, the vesicle membrane fuses with the cell membrane, a process that depends on membrane fluidity.

2.9.2 Endocytosis

As a consequence of constitutive and regulated secretion of substances from the cell, a large amount of vesicle membrane is incorporated into the cell membrane. Obviously there must be a reverse process that returns membrane material into the cell; otherwise the cell's surface area would go on increasing! In fact, the reverse process, endocytosis of the cell membrane, occurs all the time (i.e. it is constitutive), thereby keeping the membrane structure in good working order and enabling frequent adjustments to the array of receptors, carrier and channel proteins that it supports. It also means that new fatty acids can be incorporated into membrane lipids.

This sort of endocytosis is referred to as **pinocytosis** (meaning 'cell drinking') since extracellular fluid is also taken into the cell as the vesicles are pinched off from the cell membrane (Figure 2.26). In cultured fibroblasts, pinocytosis occurs at a rate estimated at 2500 vesicles per minute. The vesicles are derived from invaginations, or 'pits', in the cell membrane which are surrounded by a cage consisting of molecules of a small peripheral membrane protein called clathrin (Figure 2.27). The clathrin cage appears to stabilise the formation of the pits. As soon as a clathrin-coated vesicle has been pinched off, it loses its coat, and can then fuse with other intracellular vesicles called early endosomes.

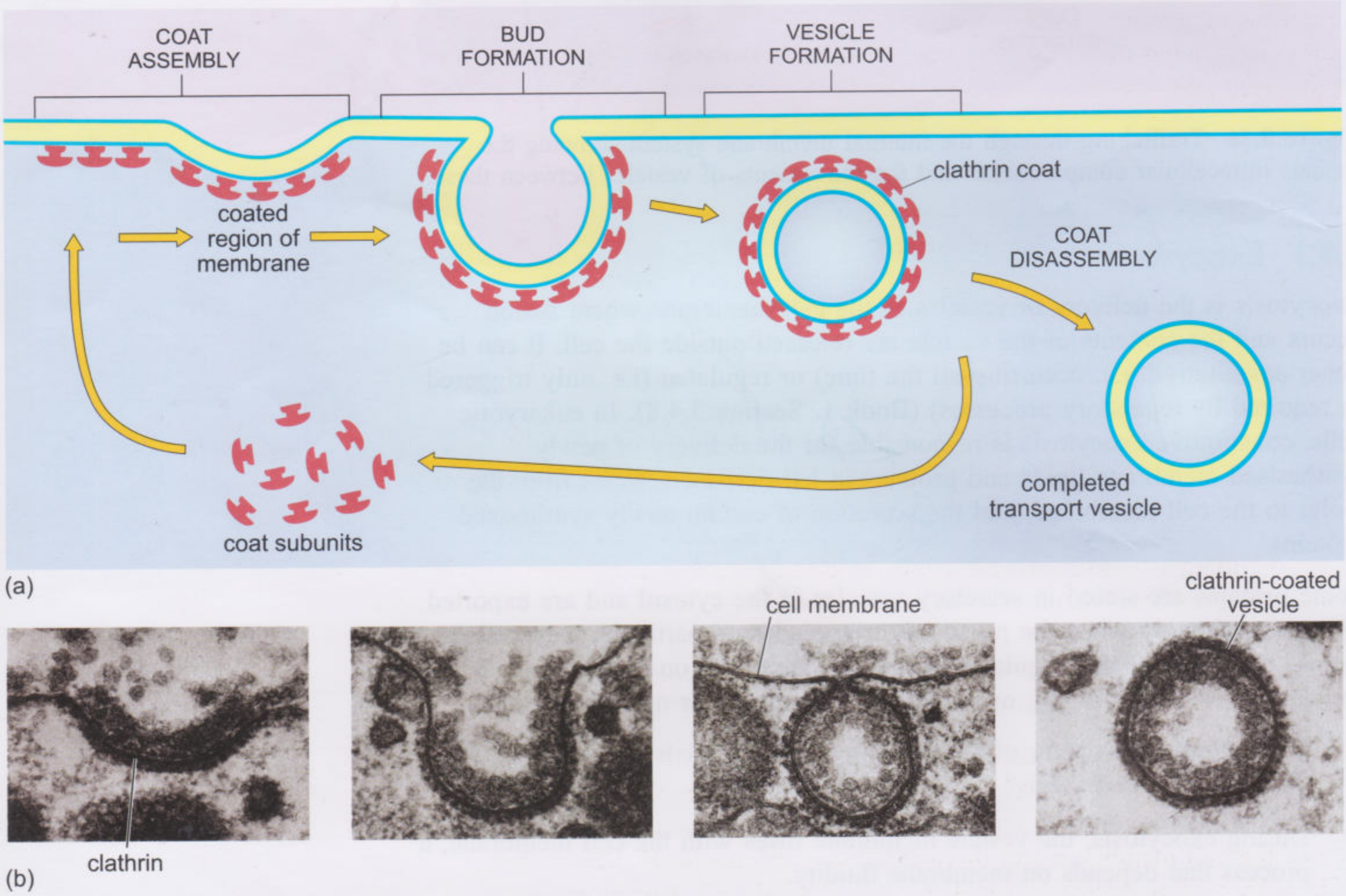


Figure 2.27 (a) Sequence of events in the formation of a clathrin-coated vesicle, such as occurs in pinocytosis, followed by loss of the clathrin coat. (b) Electron micrographs showing clathrin-coated vesicle formation.

The possible fates of early endosomes are shown in Figure 2.26. Some generate vesicles which return material directly to the cell membrane by exocytosis, while others develop into late endosomes, from which material is directed either to lysosomes (where it is digested) or recycled back to the Golgi.

Receptor-mediated endocytosis (RME) is a particular kind of endocytosis by which macromolecules and macromolecular complexes that are not transported across membranes via carrier or channel proteins can be taken up by the cell in a selective manner. RME, as the name implies, involves specific receptor proteins at the cell surface that recognise and bind the macromolecular ligand. The occupied receptors become clustered together in clathrin-coated pits and these are pinched off from the membrane into the cytosol, i.e. taken up by endocytosis. Thus, RME gets around the problem of passing the molecules to be transported through the membrane bilayer by transporting the ligand and the receptor together with part of their surrounding membrane into the interior of the cell. Concentration of the ligand in the coated pits means that a specific molecule can be taken into the cell in large amounts without also taking in a large volume of fluid. An example of RME is the uptake of low-density lipoproteins (LDLs) by animal cells. LDLs are large macromolecular complexes of protein, phospholipid and cholesterol molecules that transport cholesterol around the body. The stages in RME of LDLs are represented in Figure 2.28 and described below.

The LDL particles bind to LDL receptors at the cell surface (stage 1 in Figure 2.28) and are endocytosed in clathrin-coated vesicles (2) which, once the clathrin coat has been disassembled (3), fuse with early endosomes (4). In the endosomes, the LDLs are separated from the LDL receptors (5). The free LDL receptors are recycled back to the cell membrane (6) while the LDLs are directed to the lysosomes (7). In the lysosomes, LDLs are broken down by enzymes, producing amino acids, fatty acids and cholesterol (8), and thereby releasing cholesterol for use by the cell.

In some types of cell (e.g. mammalian epithelial cells and smooth muscle cells), there is an alternative mechanism of receptor-mediated endocytosis that does not involve clathrin. Ligands are thought to bind to receptors located in caveolae (a particular type of lipid raft, described in Section 2.6), which undergo endocytosis to form endocytic vesicles. Caveolae, and the endocytic vesicles derived from them, contain a structural protein called caveolin, which is a multipass transmembrane protein. Though caveolin is quite different from clathrin (which is a peripheral protein), it is thought that it may have a similar role to that of clathrin in stabilising the vesicle.

Certain specialised eukaryotic cells engulf and subsequently digest particulate matter by a particular kind of endocytosis known as **phagocytosis**. Phagocytosis is similar to pinocytosis in that it involves invagination of an area of the cell membrane and the formation of a vesicle. However, the vesicles formed are much larger. Their size (and shape) depend on the dimensions of the particle being ingested. So-called phagotrophic protists and some animal cells engulf particles of food (and expel the indigestible remains), and nearly all animals have phagocytic cells capable of engulfing invading microbes as well as dead cells and cell debris. In vertebrates, phagocytosis of microbes is triggered in phagocytic cells (called macrophages) when they bind to sugars or lipids on the surface of the microbes via

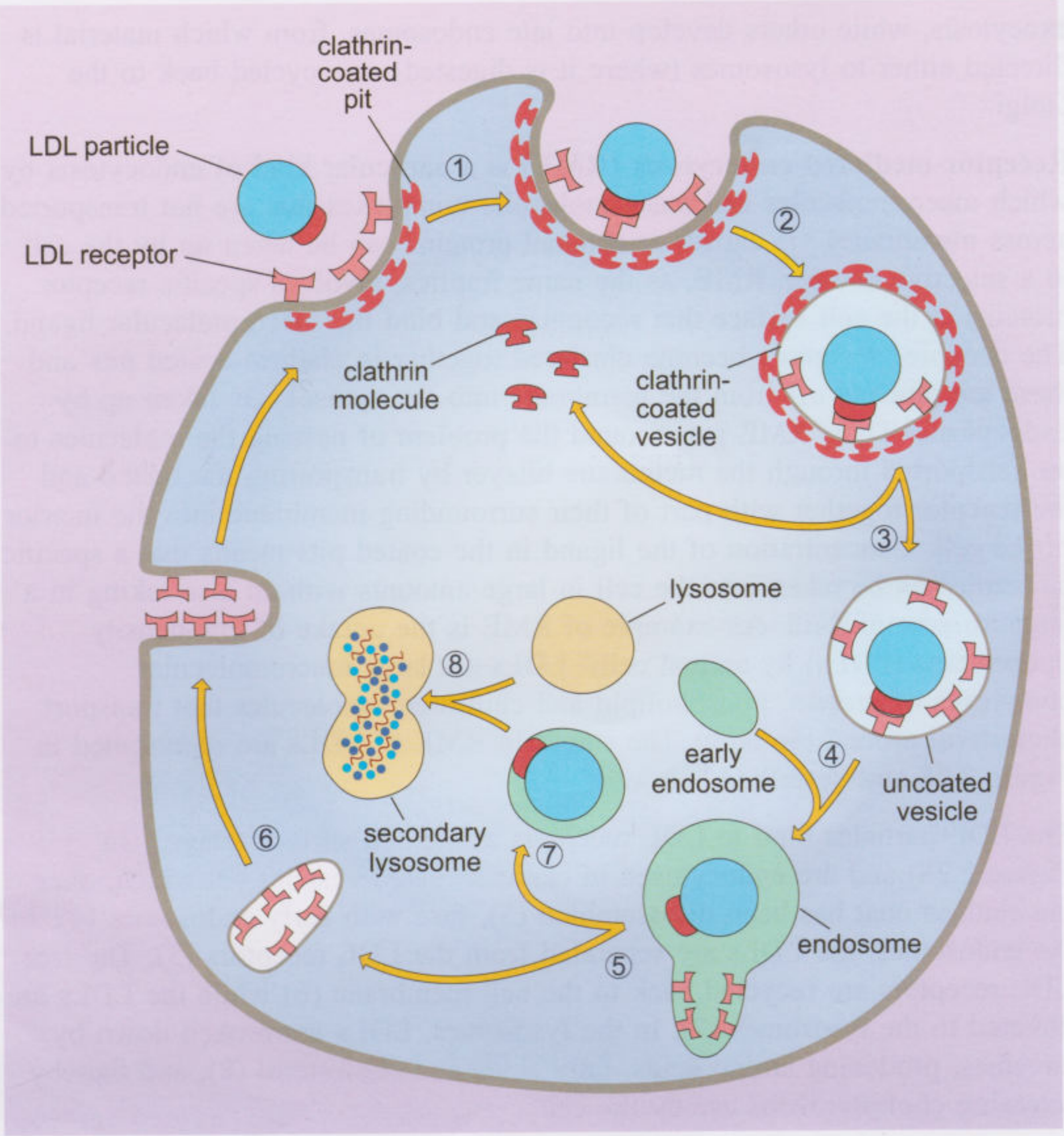


Figure 2.28 Receptor-mediated endocytosis of LDLs. After a sequence of several steps (described in the text), the LDL particle ends up in a lysosome, where enzymes release the cholesterol, which then diffuses out into the cytosol.

specialised cell-surface receptors. Whatever the nature of the ingested material, after phagocytosis, it is subjected to intracellular digestion within lysosomes and the digestion products are released into the cytosol.

■ Lysosomes have an important role in all eukaryotic cells, not just those that ingest extracellular materials. Can you recall, from Chapter 3 of Book 1, what lysosomes do?

□ They dispose of worn-out organelles (Book 1, Section 3.4.9).

Interestingly, phagocytosis and endocytosis have been shown experimentally to be affected by changes in the lipid composition of the participating membranes. Thus, by enriching the membranes of cultured macrophages with unsaturated fatty acids, and hence increasing membrane fluidity, the phagocytic activity of the cells (e.g. the ingestion of a bacterium) is increased. Conversely, endocytic activity is *reduced* in macrophages that are enriched in saturated fatty acids.

Summary of Section 2.9

- Cells secrete substances by exocytosis and take in macromolecules by endocytosis.
- There is membrane trafficking out to and inwards from the cell membrane, as well as internal two-way trafficking, via the internal membrane system.
- Membrane is recovered from the cell membrane by a type of endocytosis known as pinocytosis, in which clathrin-coated pits are pinched off (along with some extracellular fluid) to form vesicles that are trafficked through the endomembrane system.
- Receptor-mediated endocytosis (RME) is a means by which macromolecules and macromolecular complexes that are not transported across membranes via carrier or channel proteins can be taken up by the cell.
- Phagocytosis is a specific type of endocytosis that occurs in certain protists and specific phagocytic eukaryotic cells, where the cell engulfs and digests solid particles (food or foreign bodies such as microbes).

2.10 Final word

Membranes are key players in the regulation of the cell's internal environment, communication and interaction with other cells and with the extracellular environment, processing and transport of materials, and (as you will see in the following chapter) energy transduction. Chapter 4 expands on the diverse intercellular signalling processes that occur at the cell membrane, which have been briefly mentioned in this chapter.

Understanding the structural organisation of membranes and their chemical composition helps to explain how and why membranes do what they do. With recent advances in our understanding of membranes and refinements to long-standing models, it is clear that there is still much to learn about these fascinating and versatile cell structures.

2.11 Learning outcomes

2.1 Describe the structure and characteristics of biological membranes, including the fluid-mosaic model and the more recent concept of lipid rafts.

2.2 Outline the functions of biological membranes.

2.3 Relate the physical properties of biological membranes to their chemical composition and their biological functions.

2.4 Describe, using examples, the different types of membrane lipids and membrane proteins.

2.5 Explain how the chemical environment within the lipid bilayer dictates the structure of membrane proteins.

2.6 Describe the different ways in which substances can cross the cell membrane.

2.7 Explain the factors determining water movement across membranes.

2.8 Explain how ion gradients across the cell membrane result in the membrane potential.

2.9 Explain what is meant by passive diffusion, facilitated transport and active transport.

2.10 Outline how different substances are transported within a cell via the secretory pathway and how extracellular substances are taken up by the cell by endocytosis.

References

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Chapter 3 Capturing energy

3.1 Introduction

This chapter deals with a fundamental property of all living things – the ability to extract energy from their surrounding environment and to use this energy to sustain life. As you will see, a variety of strategies are employed by different organisms to ensure a continuous energy supply, and the diversity evident in these strategies has enabled organisms to exploit a variety of energy sources and environments.

Metabolism is the sum of all the chemical reactions that occur in a living organism or cell. The chemical changes that occur in living cells are incredibly varied, numerous and complex, involving the interconversion of many different chemicals (known as metabolites) in series of reactions known as **metabolic pathways**.

■ From your studies so far, what particular cellular protein components are needed to facilitate metabolic reactions?

□ Enzymes are required to catalyse metabolic reactions, ensuring that they occur at adequate rates in physiological conditions (Section 1.8).

Activity 3.1 Accessing and exploring a metabolic process chart

(LO 3.3) Allow 15 minutes in the first instance

This activity provides a link to an online metabolic process chart. You should access this chart and try exploring it a little. This activity should give you a sense of the complexity of cellular metabolism and help you to appreciate the huge number of biochemical processes that are active in cells.

Note that the chart has a search facility and you may be interested to revisit it later and search for some of the metabolites that you encounter in this chapter, to see some of the different pathways in which they are intermediates.

Metabolism is a subject that is often perceived as difficult by students; so you will be relieved to hear that you are not expected to learn the details of individual metabolic pathways. In this chapter, the focus is on fundamental principles of metabolism and a very limited number of metabolic pathways are used to illustrate these principles. The chapter begins by introducing some basic concepts that are essential to know about in order to understand metabolism.

3.2 Building order in living things – some important concepts

To begin with, consider the question: why is energy required to sustain life? It is a very particular and remarkable characteristic of living things that they are able to create and maintain order; and this is evident in every individual living cell, as well as in organisms as a whole. In contrast, when an organism dies it starts to decay; and indeed it is the nature of non-living things that, with time, they spontaneously tend towards disorder.

■ Consider what you have learnt already about the composition, organisation and structure of cells. Give some examples that illustrate their ordered nature.

□ You may have thought of, among other things: the cell membrane, which presents a barrier between the outside and the interior of the cell; intracellular compartments, which separate cellular components and processes; the synthesis of macromolecules from smaller molecules; the organisation of cell membranes; the transport of substances into and out of cells.

How does a living cell manage to generate and maintain this order? It does so by obtaining energy from the environment and converting this energy into a form that it can use to create order. As an analogy to help you understand this concept, think of how you need to expend energy to tidy up a messy room.

There are four classic laws of thermodynamics to which all matter in the Universe is subject. They are identified as the zeroth, first, second and third laws.

In Section 1.2.1 of Book 1, you were briefly introduced to the first law of thermodynamics. Thermodynamics is the study of energy, and energy can be defined as *the ability to do work or produce change*. The first law of thermodynamics states that energy is conserved – it can be neither created nor destroyed, but it can change from one form to another. The metabolic processes in a cell convert chemical bond energy released from the breakdown of food molecules into chemical bonds in newly synthesised molecules, with some of the energy escaping as heat energy.

The second law of thermodynamics describes the tendency of things to become disordered, and states that disorder (quantified by a measure known as **entropy**) can only increase in the Universe. Thus every energy transfer or transformation increases the entropy of the Universe.

How then can living things generate order without contravening this physical law? The answer is that the order that is created in the cell, using energy obtained from the environment, is more than offset by a consequent increase in disorder in the surrounding environment. This increase in disorder is achieved through the release of heat energy generated by metabolic processes. The heat energy released causes an increase in the movement of the surrounding molecules, making them more disordered; hence there is a net increase in disorder in the Universe as a whole (Figure 3.1).

As well as satisfying the second law of thermodynamics, the generation of heat by metabolic processes can be an important aspect of temperature regulation in some animals. Note too that some metabolic processes generate different forms of energy, such as light, in the case of bioluminescent cells, or mechanical energy (movement), e.g. flagellar movement in bacteria or muscle cell contraction in animals.

Both the energy used to generate order in the cell, and the heat energy released by the cell, derive from chemical reactions, which are collectively

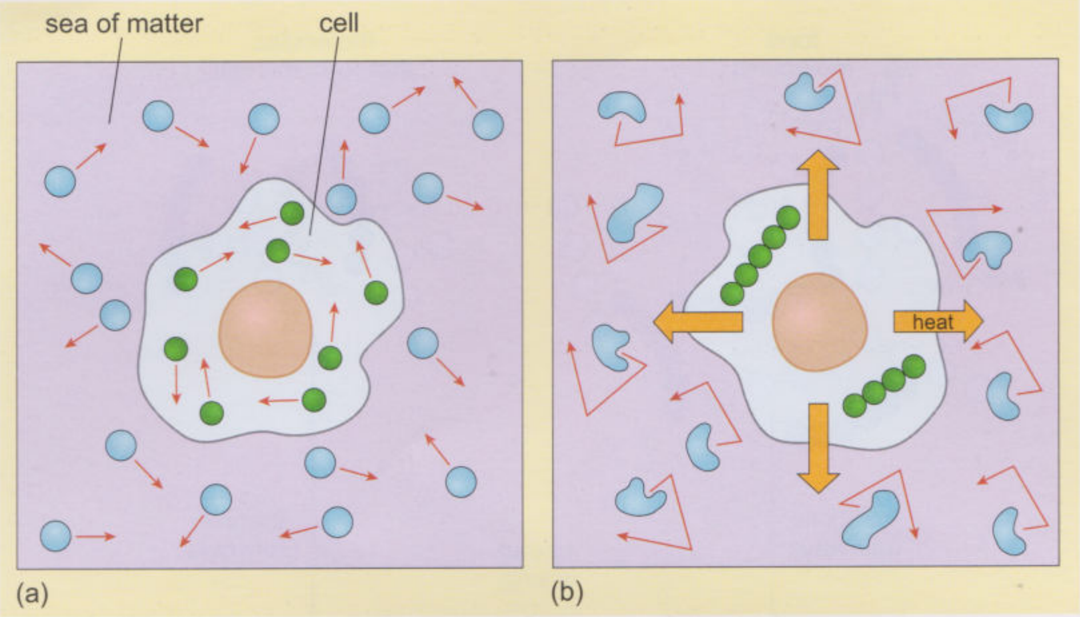


Figure 3.1 Illustration of how a cell can become more ordered (e.g. by synthesising a single large protein from many individual amino acids) while satisfying the second law of thermodynamics. In (a), molecules in both the cell and the surrounding matter (i.e. the Universe) are disordered to some extent, as represented by the arrows. As represented in (b), when the cell takes in energy from its surroundings (e.g. by metabolising foodstuffs), some of this energy is used to order the molecules within the cell (i.e. build the protein) and some is released as heat (yellow arrows). The heat energy released by the cell causes the molecules in the surrounding environment to become more disordered (represented by the jagged arrows) and undergo increased molecular movements.

described as **catabolism**. Catabolic processes break down foodstuffs (organic molecules) into smaller molecules. The energy released as a consequence of these reactions is harnessed to drive **anabolism**, the synthetic processes that build the molecules of which cells are comprised, from smaller components. Some anabolic pathways synthesise macromolecules specifically in order to store chemical bond energy. Such energy-rich macromolecules include lipids, and the carbohydrates starch and glycogen. Together, catabolic and anabolic processes constitute the cell's metabolism (Figure 3.2).

Since anabolic reactions and pathways involve an increase in molecular order, with the synthesis of cellular components from smaller molecules, they require an input of energy, i.e. they are energy-requiring or **endergonic**. By contrast, catabolic pathways and reactions, which involve a decrease in molecular order, with large molecules being broken down into smaller ones, and energy being liberated in the process, are described as **exergonic** (energy-liberating).

The terms endothermic and exothermic describe processes in which the energy produced (exothermic) or consumed (endothermic) is in the form of heat. Since metabolic reactions often involve other kinds of energy (light, chemical), the terms exergonic and endergonic are more appropriate.

Note that as well as supplying energy for anabolic processes, catabolism provides a source of the small molecule building blocks from which cell components are built.

Exergonic reactions do not require an input of energy and, in theory at least, can occur spontaneously. For biologists, the release of energy by exergonic reactions is recognised as a decrease in a quantity known as the **free energy**, denoted *G*, which is the total amount of energy in a system that can be used

Gibbs free energy, *G*, is so called after Josiah Willard Gibbs, an American mathematical physicist, who defined the term in 1878.

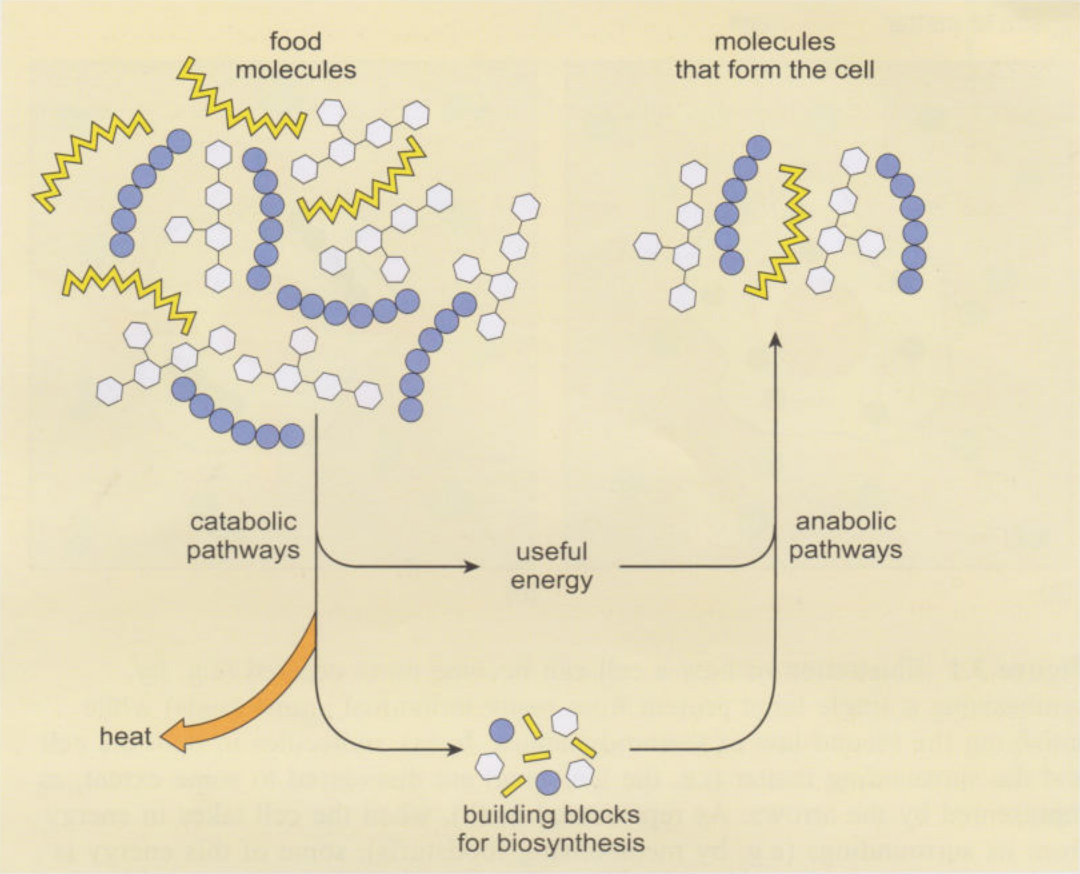


Figure 3.2 Metabolism is the combination of catabolic and anabolic processes. Catabolic processes convert food molecules into smaller molecules which can be used in anabolic processes to build new molecules needed by the cell. The energy derived from catabolism is harnessed to drive anabolism, with some being dissipated as heat.

to do work. For a particular reaction, the *change* in G, denoted ΔG (delta G), is a measure of the amount of energy released and hence the amount of disorder created in the Universe when that reaction occurs. A negative ΔG value for a reaction indicates an overall decrease in the free energy of the molecules involved in the reaction and hence an increase in disorder. By definition, therefore, exergonic reactions have a negative ΔG and endergonic reactions have a positive ΔG. Exergonic reactions are commonly described as *energetically favourable*, while endergonic reactions are *energetically unfavourable* (because energy must be put in to make them happen).

The reactions involved in synthesising cellular components (i.e. anabolic processes) are energetically unfavourable (have a positive ΔG). These reactions can only occur if they are **coupled** to energetically favourable reactions with a negative ΔG that is large enough to offset the positive ΔG of the unfavourable reaction. In other words, the ΔG of the entire process is negative. The principle of coupling a reaction that is energetically unfavourable to one that is energetically favourable will be explored in more detail with specific examples in Section 3.4.

You will recall from Chapter 1 that cellular reactions are catalysed by enzymes. An important point to grasp is that *an enzyme cannot make an energetically unfavourable reaction energetically favourable*, i.e. it cannot change ΔG for the reaction. Enzymes can only lower the energy barrier presented by the reaction, as described in Section 1.8. Put simply, enzymes can speed up reactions, but they cannot make a reaction occur that would not occur on its own.

■ Looking back at Figure 1.17, which shows the change in energy for the conversion of A into B, ΔG for the conversion is the difference between the free energy of B and that of A. Is this reaction endergonic or exergonic?

□ The reaction is exergonic, as there is a decrease in the free energy with conversion of A into B (ΔG is negative).

■ Theoretically, exergonic reactions (with a negative ΔG) should be spontaneous. In reality, although they are energetically favourable, some exergonic reactions do not readily occur in the absence of a catalyst (such as an enzyme). Looking at Figure 1.17, why is this so?

□ Even though the reaction is exergonic, for it to occur, reactant A needs to go through a high-energy transition state, i.e. an input of energy is required. The more energy that is required to reach the transition state, the fewer molecules of A will attain this state, and the slower will be the conversion of A into B. The enzyme reduces the energy required to reach the transition state and therefore the conversion of A into B occurs at a faster rate.

■ Would the enzyme affect the rate at which the reverse reaction occurs (i.e. the conversion of B into A)?

□ Yes, because the energy barrier is also reduced for the reverse reaction.

Having introduced basic concepts in metabolism, the rest of this chapter focuses on how living things capture and use the energy that they need to build order and maintain life.

Summary of Section 3.2

- Metabolism is the sum of all the chemical reactions that occur in a living organism or cell and which are necessary to sustain life. Catabolism breaks down organic molecules or other energy sources into smaller molecules. Anabolism uses energy derived from, and the small molecules produced by, catabolism to build the molecules of which cells are composed.
- The second law of thermodynamics states that disorder (entropy) can only increase in the Universe. Some of the energy released by catabolic processes is discharged as heat and there is a net increase in entropy, as demanded by this law.
- The energy taken in or liberated in a reaction represents a change in the free energy (G) of the system, ΔG. Anabolic reactions are endergonic (have a positive ΔG) and are thus energetically unfavourable; catabolic reactions are exergonic (have a negative ΔG) and so are energetically favourable.

- Energetically unfavourable reactions can only occur if they are coupled to energetically favourable reactions.
- Enzymes speed up reactions by lowering the energy required to attain the transition state. They cannot make an energetically unfavourable reaction energetically favourable; i.e. they do not change ΔG for the reaction (as this depends solely on the difference between the free energy of the product and the free energy of the substrate).

3.3 Energy sources

Organisms can be classified according to the source of energy on which they rely to fuel the synthesis of their components and other cellular activities. **Phototrophs** ('light-feeders') obtain the energy they need from the Sun. They use a variety of pigments (chemicals that can absorb light energy) to capture energy from sunlight and they convert this light energy into chemical energy in the form of ATP, which they then use in anabolic reactions to synthesise organic molecules. This whole process is known as photosynthesis.

ATP (adenosine 5′-triphosphate) is the universal form of energy used by living cells and was introduced in Section 1.2.1 of Book 1. You will learn more about ATP and other important 'energy carriers' in Section 3.4 of this chapter.

Most phototrophs, including plants, algae, cyanobacteria and many other photosynthetic prokaryotes, are able to use the ATP that they make by photosynthesis to fuel the synthesis of all their organic molecules from carbon dioxide. Such phototrophs are known as **photoautotrophs**. The organic molecules that photoautotrophs synthesise are either catabolised as a source of energy when it is dark and it is not possible for the photosynthetic organism to make ATP by photosynthesis, or they are used in anabolic processes. Certain phototrophic bacteria cannot use carbon dioxide to make their organic molecules, but instead obtain their carbon in the form of organic molecules from their surroundings; these organisms are known as **photoheterotrophs**.

Chemotrophs ('chemical-feeders') obtain energy for cellular activities by breaking chemical bonds in organic or inorganic molecules obtained from their environment. Note that in the absence of light, phototrophs must function as chemotrophs, and multicellular plants have both dedicated chemotrophic cells, such as the root cells, and cells, such as leaf cells, that can be phototrophic or chemotrophic depending on whether it is light or dark.

Among the chemotrophs, there are a few bacterial species that use inorganic ions or inorganic molecules such as hydrogen sulfide (H_2S) or hydrogen gas (H_2) as a source of energy, and use carbon dioxide (CO_2) as a source of carbon. These organisms are described as **chemoautotrophs**. However, the vast majority of chemotrophs (including all animals, fungi, protists and many prokaryotes) use a variety of organic molecules from other living or dead organic matter obtained in their diet as a source of both chemical energy and carbon. These organisms are known as **chemoheterotrophs**.

All organisms thus belong to one of four different groups according to their energy source and carbon source, as summarised in Table 3.1. Photoautotrophs and chemoheterotrophs together comprise the vast majority of species.

Table 3.1 Classification of organisms according to their sources of energy and carbon.

		Energy source		
		Light	**Inorganic molecules or ions**	**Organic compounds**
Carbon source	**Carbon dioxide**	**Photoautotroph** e.g. photosynthetic prokaryotes such as cyanobacteria; plants; algae	**Chemoautotroph** e.g. certain prokaryotes such as *Sulfolobus* and the methanogens	
	Organic compounds (in the diet)	**Photoheterotroph** e.g. some prokaryotes such as *Rhodobacter*		**Chemoheterotroph** e.g. animals, fungi, protists, many prokaryotes

This chapter focuses primarily on photoautotrophs (which include plants) and chemoheterotrophs (which include animals). However, there is also brief consideration of some organisms that use other sources of energy and carbon, to illustrate the metabolic diversity and the variety of strategies that have evolved to enable organisms to capture and use energy. To appreciate the processes by which organisms obtain and use energy, it is helpful if you understand certain fundamental chemical principles, outlined briefly in Box 3.1.

Box 3.1 Basic chemical principles and concepts in metabolism

Hydrolysis reactions

A hydrolysis reaction is a reaction in which a covalent bond is broken by addition of a water molecule. Hydrolysis reactions are common in biological systems: for example, when polymers such as polypeptides, carbohydrates or nucleic acids are catabolised to their constituent monomers. Hydrolysis of the bond between two monomers within a polymer chain entails the splitting of a water molecule to form a proton (H^+) and a hydroxyl ion (OH^-), each of which are donated to one of the products, as represented in the equation below:

$$A\text{–}B + H_2O \rightarrow A\text{–}H + B\text{–}OH$$

The reverse of the hydrolysis reaction, termed condensation, generates a water molecule and is the reaction that occurs when macromolecules are synthesised from their constituent monomers:

$$A\text{–}OH + B\text{–}H \rightarrow A\text{–}B + H_2O$$

Another example of a hydrolysis that is crucial to metabolism is the hydrolysis of ATP (Section 3.4) to adenosine 5′-diphosphate (ADP) and inorganic phosphate (P_i), releasing the energy stored in the terminal phosphate bond of ATP (Figure 3.3). ATP hydrolysis is an energetically favourable reaction that is often used to 'force' a coupled energetically unfavourable reaction to occur.

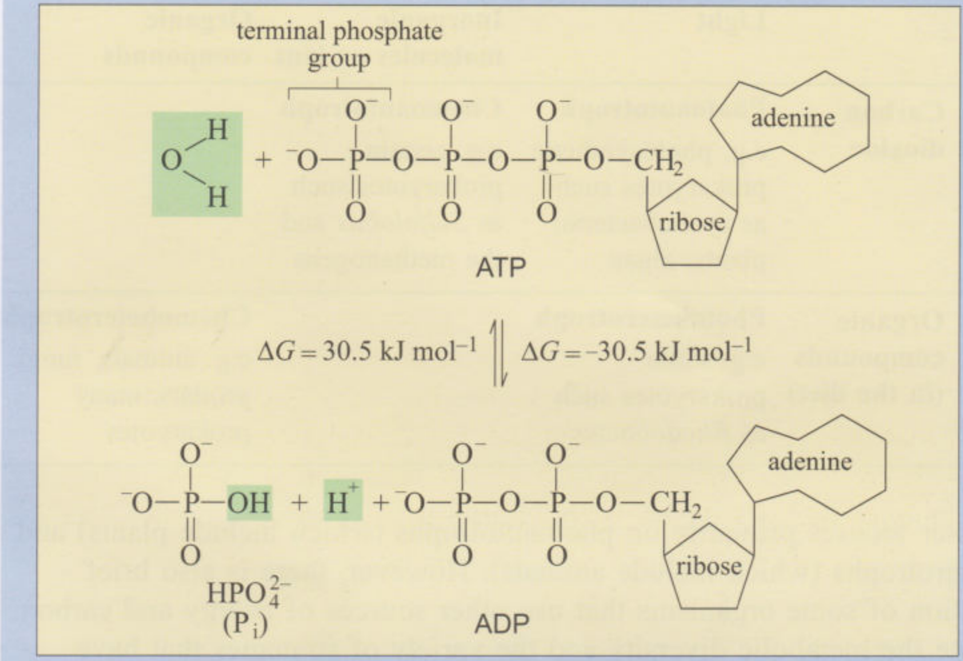
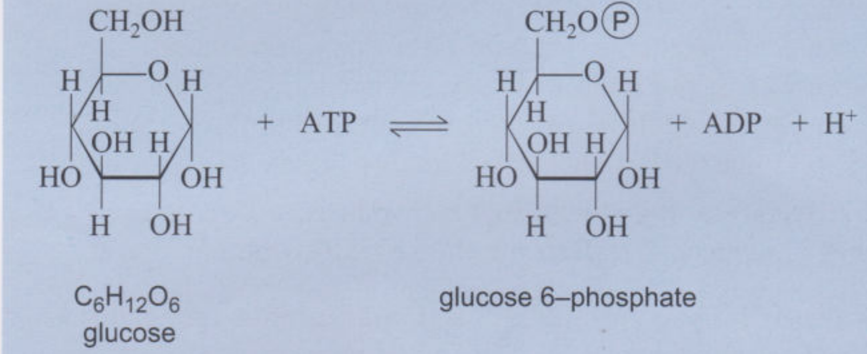

terminal phosphate group
adenine
ribose
ATP
$\Delta G = 30.5$ kJ mol^{-1}
$\Delta G = -30.5$ kJ mol^{-1}
HPO_4^{2-}
(P_i)
ADP

Figure 3.3 The hydrolysis of ATP to ADP and P_i is highly exergonic and therefore energetically favourable.

Notice that in Figure 3.3, ΔG is given in units of kJ mol^{-1}, which means kilojoules per mole of ATP converted by this reaction into ADP. Joules or kilojoules (1 kJ = 1000 J) is the standard unit for energy.

Reversible reactions and chemical equilibrium

Theoretically all chemical reactions are reversible, as denoted by the opposing half-arrows in Figure 3.3 and in the reaction below:



Thus the products of the forward reaction (left to right) are the reactants in the reverse reaction (right to left). This particular reaction is one of the steps of glycolysis, which you will encounter in Section 3.6.1.

■ You may have noticed that this reaction couples the exergonic conversion of ATP into ADP with addition of the free phosphate group to glucose. The overall forward reaction, as represented above, requires a large input of energy, i.e. it is highly exergonic. What can you say about ΔG for the reverse reaction?

□ It is positive; i.e. the reverse reaction is energetically unfavourable.

Regardless of whether you start with glucose and ATP or glucose 6-phosphate and ADP, in the reversible reaction shown above, the components could *in theory* reach what is known as chemical **equilibrium**, where there is a mixture of all components and there is no net change in the levels of the different components. However, equilibrium is only achieved in an isolated system, where there is no input of reactants or removal of products. Living cells are *not* isolated systems. There is a constant flow of materials into and out of the cell and metabolic reactions never reach equilibrium. Reactants (in this example, glucose derived from nutrients) are constantly entering the reaction, and products (in this example, glucose 6-phosphate) are used up by the next step in the metabolic pathway. In this way, reactions are 'pulled' in one direction.

Metabolic reactions are therefore typically written down as if they were irreversible, with a single unidirectional arrow, as shown below:

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{ADP} + \text{H}^+$$

Oxidation and reduction

The chemical reactions involved in releasing energy from chemical bonds are of a type that chemists call **oxidation**, which means that electrons are removed from the reactant. In biological systems, oxidation generally involves the removal of hydrogen atoms (i.e. a proton plus an electron) and the addition of oxygen atoms to the molecule. The reverse of oxidation is **reduction**, which is the addition of electrons to a molecule and usually involves addition of hydrogen atoms and loss of oxygen atoms. In general, oxidation reactions release energy – they are exergonic. Reductions are the reverse of oxidation reactions and hence require an input of energy. They are therefore endergonic.

Oxidation and reduction always occur together; that is, if one reactant is oxidised, another is reduced. To illustrate this, consider the reaction in Figure 3.4, the overall reaction for the complete oxidation of the simple sugar glucose ($C_6H_{12}O_6$) in the presence of molecular oxygen (O_2). Glucose is one of the most important sources of chemical energy utilised by many chemotrophs. As illustrated in Figure 3.4, the oxidation of glucose molecules is accompanied by reduction of O_2 molecules to form H_2O.

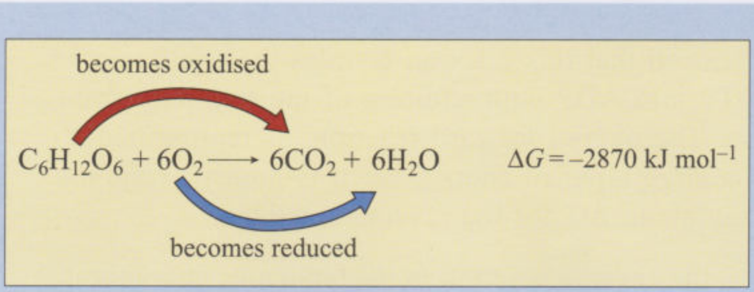

becomes oxidised
$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$
$\Delta G = -2870\ \text{kJ mol}^{-1}$
becomes reduced

Figure 3.4 Oxidation of glucose is accompanied by reduction of O_2 in an exergonic redox reaction.

Remember, *oxidation is exergonic* while *reduction is endergonic*. In the reaction represented in Figure 3.4, the energy produced on oxidation of glucose exceeds that used to reduce O_2 molecules to H_2O and there is therefore a net release of energy. If glucose were to be burned in the presence of O_2, this is the reaction that would occur but the energy would all be released at once in the form of heat. In biological systems, the same conversion is achieved through a series of enzyme-catalysed reactions known as **aerobic respiration**. As you will see when you take a closer look at the metabolic pathways by which glucose is oxidised, by having many small steps, the energy released is released in stages and transferred to energy carriers such as ATP or NADH (see Section 3.4), which can then transfer the energy to drive energy-requiring reactions.

Redox reactions and redox potential

Reactions that entail the oxidation of one substrate and the reduction of another are sometimes referred to as **redox** reactions (e.g. that illustrated in Figure 3.4). Like any other reaction, whether a redox reaction is energetically favourable depends on whether it is endergonic or exergonic; that is, whether ΔG for the overall reaction is positive or negative. In the case of redox reactions, this depends on the relative affinity of the reactant for electrons (i.e. how attractive it is to electrons). This can be measured and is given by a value known as **redox potential (*E*)**, effectively its potential to be reduced or oxidised. Low redox potential indicates low electron affinity, so *electrons will be readily transferred from a reactant with low redox potential to one with a higher redox potential*. The transfer of electrons from a reactant with a low redox potential to one with a higher redox potential is exergonic and energetically favourable (ΔG is negative); conversely, transfer of electrons from a reactant with a high redox potential to one with lower redox potential is energetically unfavourable (ΔG is positive). The difference in redox potential between two compounds (ΔE) is important because all electron transfer releases free energy, and ΔG is proportional to ΔE.

Summary of Section 3.3

- All organisms belong to one of four different groups according to (i) the source of energy that they use to fuel anabolism and other cellular activities, and (ii) the source of the carbon used to synthesise their carbon-containing components.
- Phototrophs use energy from the Sun to synthesise ATP. Of these organisms, the photoautotrophs (such as green plants) can synthesise all their carbon-containing compounds from CO_2, while the photoheterotrophs (which include certain prokaryotes) use organic compounds from their surroundings as a source of carbon.
- Chemotrophs obtain energy from the catabolism of organic or inorganic molecules or ions obtained from their environment. Of these organisms, the chemoautotrophs (which include certain prokaryotes) use inorganic chemicals for energy and CO_2 as a source of carbon, while the chemoheterotrophs (which include all animals, fungi, protists and many bacteria) use a variety of organic molecules obtained in their diet, as a source of both chemical energy and carbon.
- Hydrolysis reactions, in which a covalent bond is broken by addition of a water molecule, are common in biological systems. The reverse of the hydrolysis reaction, termed condensation, generates a water molecule and is the reaction that occurs when macromolecules are synthesised from their constituent monomers.
- Theoretically all chemical reactions are reversible and can reach equilibrium, where there is a mixture of all components and there is no net change in the levels of the different components. In practice, metabolic reactions never reach equilibrium because there is constant input of products and removal of reactants, so these reactions tend to go almost completely in one direction.
- In biological systems, oxidation, which is loss of electrons, generally involves the removal of hydrogen atoms (i.e. a proton plus an electron) and the addition of oxygen atoms to the molecule. The reverse of oxidation is reduction, which is the addition of electrons to a molecule and usually involves addition of hydrogen atoms and loss of oxygen atoms.
- Redox potential (E) is a measure of the affinity of a molecule or ion for electrons.

3.4 ATP and other coenzymes as energy carriers

Adenosine 5′-triphosphate (ATP; Section 1.2.1 in Book 1) plays an important role as an energy carrier in all cells and organisms, regardless of the energy source that they exploit. ATP is one of a number of different activated carrier molecules, known as **coenzymes**, which store energy derived from exergonic catabolic processes in chemotrophs, or from light in phototrophs, and release this energy as and when it is required to drive endergonic reactions (Figure 3.5). These small molecules deliver the energy that they carry to wherever it is required within the cell.

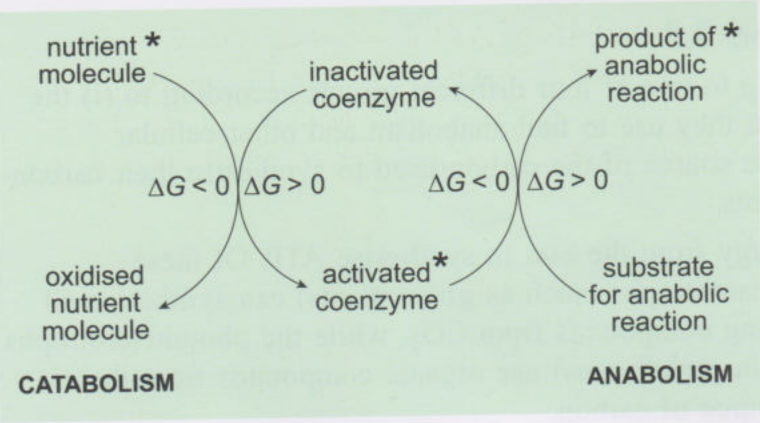


Figure 3.5 Function of coenzymes as energy intermediates in metabolism, transferring energy derived from catabolic processes to drive anabolic processes. An asterisk (*) represents the energy that is transferred in this way.

Activation of coenzymes, as represented in Figure 3.5, is achieved in one of two ways:

1 by formation of an energy-rich covalent bond, as is the case with ATP;

2 by the addition of high energy electrons (i.e. reduction), as with adenine dinucleotides (e.g. NAD^+) as described below.

ATP is ubiquitous in the cells of all living organisms and is the most abundant coenzyme. It consists of adenosine (an adenine base plus a ribose sugar molecule) to which three phosphate groups are attached in a chain, as shown in Figure 3.3. The energy released on breaking the bond linking the terminal phosphate group is considerable and the reaction is therefore highly exergonic ($\Delta G = -30.5 \text{ kJ mol}^{-1}$). The products of this reaction, which is an example of a hydrolysis reaction (see Box 3.1), are ADP and P_i.

As has already been mentioned, energetically favourable reactions, such as the hydrolysis of ATP, can be used to 'drive' reactions that are energetically unfavourable. This is achieved by effectively coupling the two reactions, as illustrated for generalised reactions below.

Energetically unfavourable (endergonic) reaction:

$$\text{A} + \text{B} \rightarrow \text{A–B} \qquad \Delta G \text{ is positive}$$

Energetically favourable (exergonic) reaction:

$$\text{C–D} \rightarrow \text{C} + \text{D} \qquad \Delta G \text{ is negative}$$

Provided the sum of the ΔG values for the two coupled reactions is negative, then the overall reaction is energetically favourable (exergonic):

$$\text{A} + \text{B} + \text{C–D} \rightarrow \text{A–B} + \text{C} + \text{D} \qquad \text{net } \Delta G \text{ is negative}$$

Coupling of reactions in this way most commonly involves both reactions being catalysed by the same enzyme.

The energetically favourable hydrolysis of ATP is coupled to many reactions that would otherwise be unfavourable. In many cases, such coupled reactions involve the transfer of the terminal phosphate group from ATP to another substrate. Examples of such *phosphate transfer reactions* are found in the initial steps in the catabolism of glucose.

- ■ What phosphate transfer reaction have you already encountered in this chapter?
- □ The conversion of glucose and ATP into glucose 6-phosphate and ADP (Box 3.1) is a phosphate transfer reaction. This is the first step in glycolysis:

  glucose + ATP → glucose 6-phosphate + ADP + H^+

Aside from ATP, many other coenzymes are involved in metabolism. Some of these serve not only as a means of transferring energy, but also as a source of chemical groups required for particular reactions (Table 3.2). For example, coenzyme A in its activated form carries an acetyl group ($-COCH_3$), which, like the terminal phosphate in ATP, is attached to the coenzyme via a high-energy covalent bond. Transfer of the acetyl group is used to add 2-carbon units in the biosynthesis of larger organic molecules.

In contrast, the adenine dinucleotides – NAD^+, $NADP^+$ and FAD (full names are given in Table 3.2) – are coenzymes that are activated when they pick up two high-energy electrons and either one (in the case of NAD^+ and $NADP^+$) or two (in the case of FAD) protons during catabolic reactions.

- ■ What kind of reaction is this?
- □ It is a reduction reaction (Box 3.1).
- ■ What kind of reaction must accompany activation of NAD^+, $NADP^+$ and FAD?
- □ An oxidation reaction (since reductions are necessarily accompanied by an oxidation reaction; see Box 3.1).

Thus activation (reduction) of NAD^+, $NADP^+$ or FAD requires simultaneous oxidation of a substrate molecule. In turn, transfer of the high-energy electrons and proton(s) from the activated coenzymes reduces substrates in anabolic reactions, driving energetically unfavourable reactions. Thus these coenzymes are reduced in catabolic reactions and oxidised in anabolic reactions, effectively cycling between their oxidised and reduced states and transferring energy and what can be thought of as 'reducing power' (Figure 3.6).

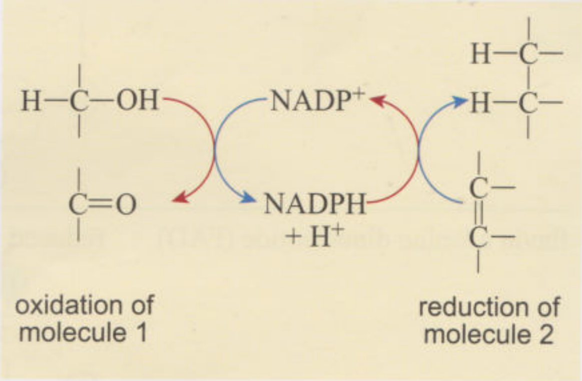
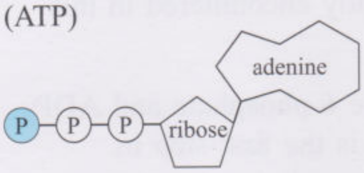
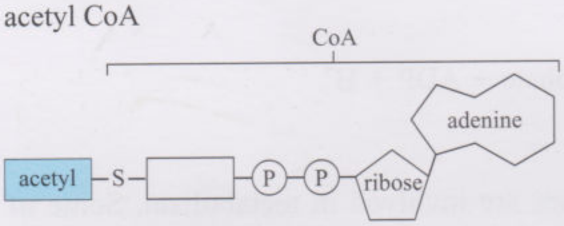
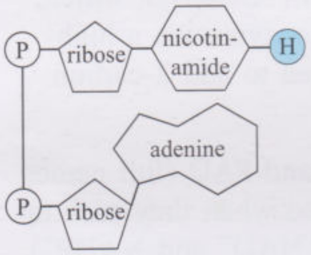
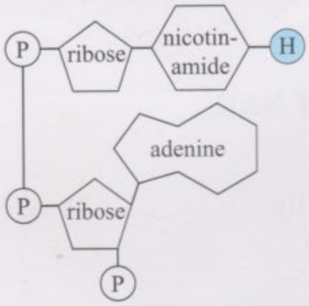
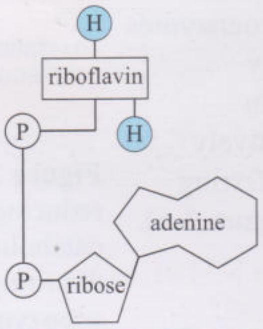


Figure 3.6 The transfer of energy and reducing power from energy-yielding catabolic reactions to drive energy-requiring anabolic reactions is facilitated by coenzymes such as $NADP^+$, shown here cycling between its oxidised and reduced states.

Table 3.2 Coenzymes commonly used in metabolism, and the group transfer reactions in which they are involved. Simplified structures, in which the transferred group is highlighted in blue, are shown in the central column. The groups that these coenzymes transfer are also highlighted in bold in the group transfer reactions listed in the right-hand column.

Coenzyme – unactivated form	**Coenzyme – activated form**	**Group transfer reaction** (transferred group in **bold**)
adenosine 5′-diphosphate (ADP)	adenosine 5′-triphosphate (ATP) 	$\mathrm{ATP} \rightarrow \mathrm{ADP} + \mathbf{P_i}$
coenzyme A (CoA)	acetyl CoA 	*$\mathrm{CH_3CO{-}S{-}CoA} \rightarrow \mathrm{CoA{-}SH} + \mathbf{{-}CH_3CO}$
nicotinamide adenine dinucleotide ($\mathrm{NAD^+}$)	reduced nicotinamide adenine dinucleotide (NADH) 	$\mathrm{NADH} \rightarrow \mathrm{NAD^+} + \mathbf{H^+} + \mathbf{2e^-}$
nicotinamide adenine dinucleotide phosphate ($\mathrm{NADP^+}$)	reduced nicotinamide adenine dinucleotide phosphate (NADPH)  P ribose nicotin-amide H adenine P ribose P	$\mathrm{NADPH} \rightarrow \mathrm{NADP^+} + \mathbf{H^+} + \mathbf{2e^-}$
flavin adenine dinucleotide (FAD)	reduced flavin adenine dinucleotide ($\mathrm{FADH_2}$) 	$\mathrm{FADH_2} \rightarrow \mathrm{FAD} + \mathbf{2H^+} + \mathbf{2e^-}$

* Note that coenzyme A is represented sometimes as CoA and sometimes (as in this case) as CoA–SH, to draw attention to terminal sulfhydryl group (–SH), which reacts with the acetyl group in the transfer reaction that forms acetyl CoA.

It is interesting to note that the groups that coenzymes transfer are very small compared with the size of the coenzyme as a whole (e.g. acetyl CoA and NADH in Figure 3.7). The purpose of the larger part of the coenzyme molecule (ADP, NAD^+, CoA, etc.) to which the small transferable group is attached is to bind to the enzymes that catalyse the particular reactions in which the coenzyme is involved. All of the coenzymes listed in Table 3.2 contain a nucleotide – comprising a base (adenine in these particular cases), a sugar and a phosphate group. It has been speculated that their nucleotide components may be an indication that coenzymes originated at a time during evolution when biological catalysts were RNA molecules rather than proteins (discussed in Section 1.1.2 of Book 1), and the nucleotide may have been important for binding the coenzyme to RNA enzymes.

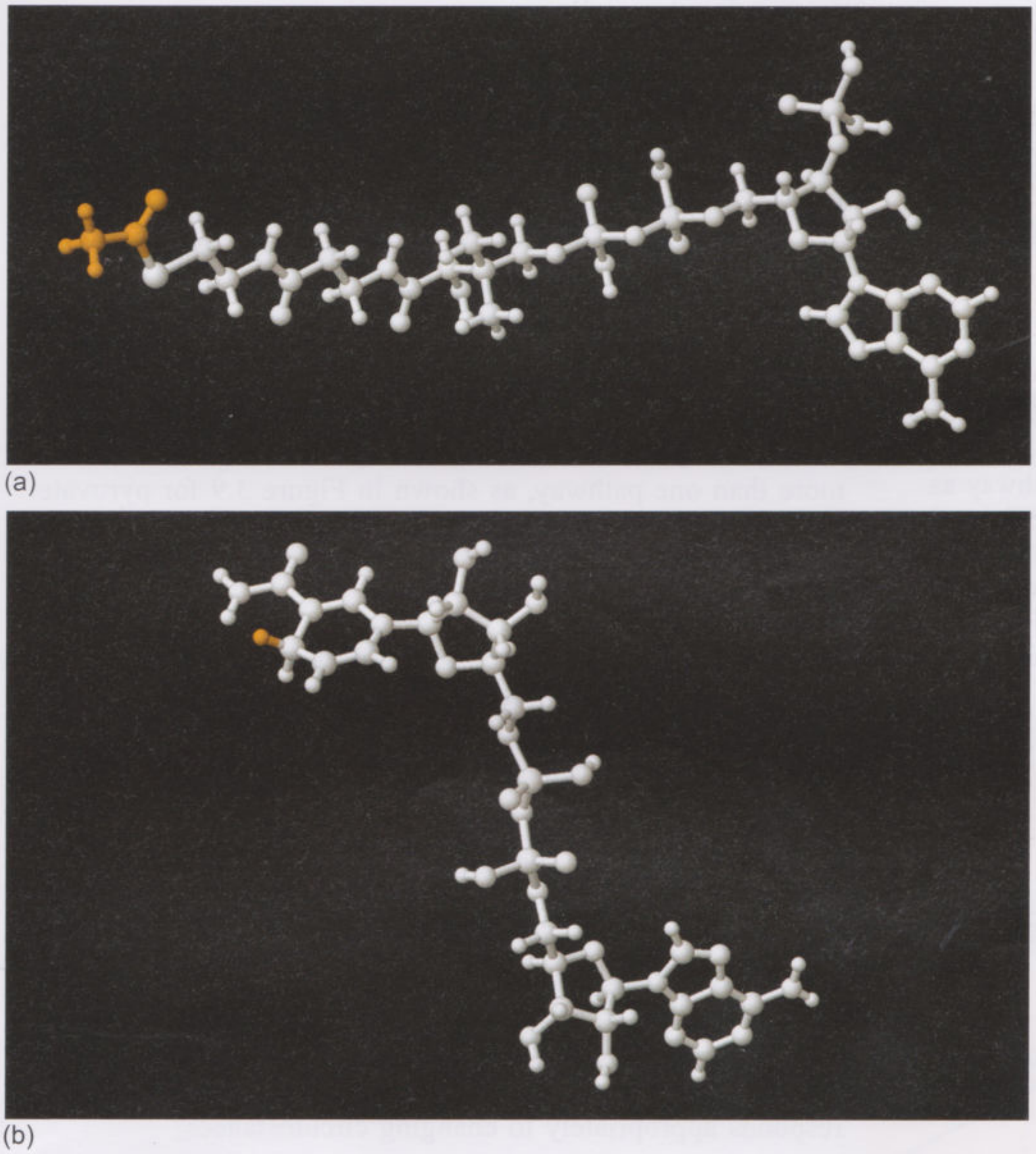


Figure 3.7 Ball and stick structures of (a) acetyl CoA and (b) NADH, in which the atoms of the transferable groups are highlighted in orange and the rest of the molecule is shown in white.

Summary of Section 3.4

- Coenzymes (which include ATP, acetyl CoA, NADH, NADPH and $FADH_2$) are molecules that, in an activated form, can store energy and release it as and when it is required for endergonic reactions. This is achieved by coupling the endergonic reaction with the conversion of the activated (high-energy) coenzyme into its inactivated state.
- As well as transferring energy, some coenzymes are a source of chemical groups required for particular reactions (e.g. a phosphate group from ATP or an acetyl group from acetyl CoA).

3.5 Metabolic pathways and their control

Cellular metabolism is actually a very complex network of enzyme-catalysed reactions, with thousands of different enzymes and intermediates involved in the metabolism of a typical cell (as will be apparent from Activity 3.1), and many reactions occurring simultaneously.

Most metabolic reactions are part of a series, a metabolic pathway, in which the product of one reaction becomes the substrate (reactant) for another reaction (represented schematically in Figure 3.8). The substrates, intermediates and products in metabolic pathways are commonly called **metabolites**.

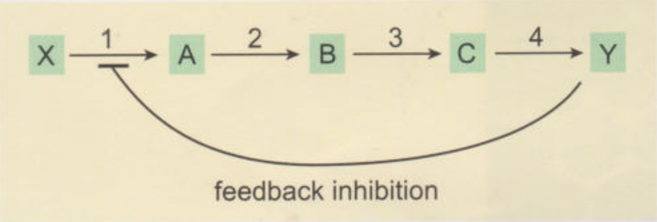


Figure 3.8 A simple representation of a metabolic pathway. Note that the pathway as shown converts X into Y via intermediates A, B and C. Each step is catalysed by a different enzyme (1–4). In this particular representation, the product Y inhibits the activity of enzyme 1 at the start of the pathway. This is an illustration of feedback inhibition.

Note that a particular metabolite may be an intermediate in more than one pathway, as shown in Figure 3.9 for pyruvate. Pyruvate, the ultimate product of glycolysis, is an intermediate in a number of other different pathways, producing a variety of products.

These include lactate, the amino acid alanine, the activated coenzyme acetyl CoA, which is used to build, among other things, fatty acids, and oxaloacetate (in a process called gluconeogenesis in which glucose is synthesised).

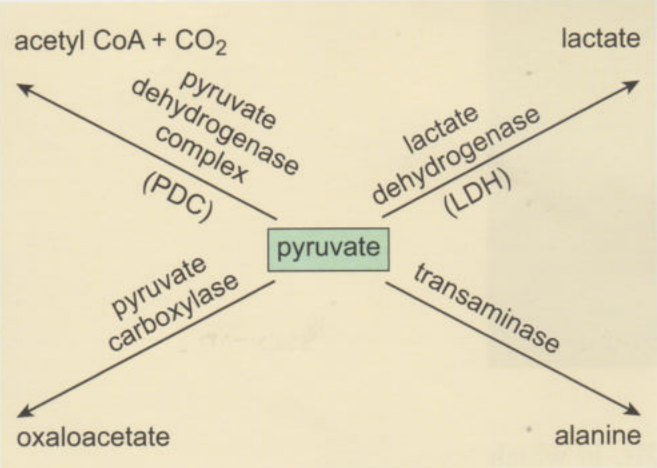


Figure 3.9 Alternative metabolic pathways in which pyruvate is an intermediate.

The rate of oxidation of foodstuffs in the cell is carefully tuned to its current needs, since there is clearly no point, for example, in breaking down valuable fuel molecules if the cell is already able to meet all its demands for ATP. The same is true of all metabolic pathways; there has to be an appropriate balance between catabolism and biosynthesis. How is this complex network of cellular metabolic reactions controlled and coordinated to ensure that the cell has what it needs and that it responds appropriately to changing circumstances?

3.5.1 Controlling enzymes

One way in which **metabolic flux**, the rate at which materials flow through a particular pathway, is controlled is through the enzymes that catalyse the various metabolic reactions.

There are two main ways in which an enzyme can be controlled. The first is by altering the amount of enzyme present, which, like any protein, depends on both the level of expression of its corresponding gene (as discussed in Chapter 6 of Book 1) and the rate at which the enzyme is degraded. The second way is by modifying the activity of the existing enzyme. In animals, alterations in the level of expression of some enzymes are mediated indirectly by certain hormones, which is a relatively slow way of controlling an enzyme compared with mechanisms that regulate the activity of the enzyme directly.

■ From your studies of proteins in Chapter 1, recall mechanisms by which enzyme activity is regulated directly.

□ Allosteric regulation and reversible covalent modifications, in particular phosphorylation, are important mechanisms by which enzyme activity can be regulated (Section 1.8.5). Some enzymes, such as the digestive enzymes pepsin and trypsin, which act extracellularly in the digestive tract of some animals, are converted from an inactive form into an active form by proteolytic cleavage (Section 1.6.2).

You should recall that in allosteric regulation, a so-called allosteric effector binds specifically to a regulatory site on the enzyme, distinct from the substrate binding site, thereby causing a conformational change in the catalytic site that affects enzyme activity. Figure 3.8 illustrates one particular way in which allosteric regulation can control metabolic flux. In **feedback inhibition**, the product of a metabolic pathway inhibits an enzyme that catalyses an earlier reaction in the pathway. In the illustration in Figure 3.8, a product of a metabolic pathway (Y) is an allosteric inhibitor of an enzyme that catalyses an earlier reaction in the pathway (enzyme 1). Thus, increased levels of the product Y cause a decrease in flux through the pathway. If levels of Y decline (because it is utilised in some other pathway), this inhibitory effect will be reduced and metabolic flux will increase. In this way, the level of Y (and that of the intermediates in the pathway) is controlled.

■ What is the net result of feedback inhibition and why might this be desirable?

□ As the product of the pathway accumulates, feedback inhibition causes its synthesis to be reduced. This ensures that the product is not produced in excess of the level required by the cell.

■ Considering Figure 3.8, why is it desirable to inhibit enzyme 1 rather than enzyme 4?

□ Inhibition of enzyme 1 at the start of the pathway, rather than enzyme 4, is a more efficient means of switching off synthesis of the product Y, as it obviates the need for the intervening reactions.

It used to be thought that certain key enzymes catalysed rate-determining steps in metabolic pathways. However, it has become clear that control is, at least to an extent, shared between all the enzymes in a pathway. While certain regulatory enzymes do often take a major share, there is usually no *single* control point as was once thought.

3.5.2 Substrate availability

A further mechanism by which metabolic flux is regulated is through substrate availability. Consider a pathway with a number of intermediates. Cells will operate in a steady state if reactants are continually being supplied and products removed; that is, if the concentration of intermediates between them remains the same. It is like a bath with the taps turned on and plug pulled out, continually filling and emptying. At a certain flow rate from the tap, the level of bath water stays the same, even though individual water molecules are being replaced. If availability of the first reactant or any of the intermediates drops, flux through the whole pathway drops. This happens when supplies of the first reactant dwindle, or when an intermediate is diverted into a branching pathway.

3.5.3 Compartmentalisation and transport of substrates

As you will see in the discussions of aerobic respiration in Section 3.6, compartmentalisation, the physical separation of metabolic processes in different cellular compartments, i.e. organelles, is a feature of eukaryotic cells. As a consequence, transport proteins play a major role in metabolism since without them most cell and organelle membranes would be impermeable to organic intermediates, nucleotides and ions. A pyruvate transporter, for example, is needed to move pyruvate from the cytosol into the mitochondrion so that it can be converted into acetyl CoA (in what is known as the link reaction, described in Section 3.6.2). Specific transporters are also needed for phosphate and calcium ions, and for equilibrating pools of NAD^+/NADH and ATP/ADP between the mitochondria and the cytosol. One advantage of compartmentalisation is that soluble reactants and most enzymes are confined to smaller spaces, and are hence more concentrated than if they were free to diffuse throughout the cell. Intermediates of the TCA cycle (Section 3.6.2), for example, remain within the mitochondrial matrix (although specific transporters allow some of them to participate in metabolic pathways elsewhere in the cell).

3.5.4 Metabolic channelling

Some enzymes are physically so closely linked that the product of one immediately becomes the substrate of another, passing directly between the two active sites without dissolving in the surrounding medium at all. This **metabolic channelling** is particularly advanced in the pyruvate dehydrogenase complex (PDC), the multienzyme complex that catalyses the conversion of pyruvate into acetyl CoA (in the link reaction, described in Section 3.6.2), and which has three different types of protein subunit, each catalysing a different step in the overall reaction. The electron transport chain (Section 3.6.3) is another example of metabolic channelling, where electron carriers are embedded in the inner mitochondrial membrane in order of their electron affinity (i.e. redox potential; Section 3.3). Electrons are channelled rapidly from one to another without passing into the surrounding medium.

Summary of Section 3.5

- A series of reactions in which the product of one reaction becomes the substrate for another is referred to as a metabolic pathway.
- Metabolic flux, the rate at which materials flow through a particular pathway, is controlled through the enzymes that catalyse the various metabolic reactions, by altering their expression/degradation or by modifying their activity. Availability of a substrate also influences metabolic flux.
- Feedback inhibition, in which the product of a metabolic pathway binds to and inhibits an enzyme that catalyses an earlier reaction in the pathway, is an example of an allosteric mechanism for regulating enzymes in metabolic pathways.
- Localisation of enzymes and their substrates in particular cellular compartments can increase the efficiency of particular processes. In some cases, close proximity of substrates and enzymes of a metabolic pathway means that intermediates are passed directly between enzymes in the pathway. This metabolic channelling, which may be facilitated by localisation of enzymes in membranes or association of enzymes in a multienzyme complex, can increase efficiency of the process.

3.6 Glucose oxidation in eukaryotic cells

This section looks at the processes by which all animal and plant cells (or more specifically, all chemoheterotrophic and photoautotrophic cells) obtain energy by oxidising organic molecules. Remember that while animal cells obtain these organic molecules from nutrients consumed by the organism, plant cells make their own from carbon dioxide, using energy derived from photosynthesis.

In animals, nutrients in the form of carbohydrates, proteins and fats are broken down into smaller units (respectively: simple sugars, amino acids, and fatty acids plus glycerol) by the process of digestion. This stage starts outside the cells, in the digestive system, and also occurs inside the cells in lysosomes, membrane-bound organelles that contain digestive enzymes (Book 1, Section 3.4.9). After digestion, the resulting small organic molecules are distributed around the body in the blood circulation and enter the cytosol of individual cells, and it is here that their oxidation begins.

Despite the variety of the different types of organic molecules, in all eukaryotic cells the pathways by which these molecules are broken down converge, and the ultimate synthesis of ATP occurs in the same way, regardless of the original energy source. To help you understand the steps involved in the oxidation of organic molecules in the cell, the following discussion considers the oxidation of the simple sugar glucose, which is the 'fuel' that cells most often use.

As has been already mentioned (Box 3.1), the complete oxidation of glucose to carbon dioxide and water is exergonic (ΔG for the reaction being −2870 kJ per mole of glucose oxidised; Figure 3.4).

The oxidation of glucose by the cell under aerobic conditions, i.e. in the presence of oxygen, is known as **aerobic respiration**. It is a highly efficient way in which the cell catabolises glucose to make ATP; Figure 3.10 summarises this process.

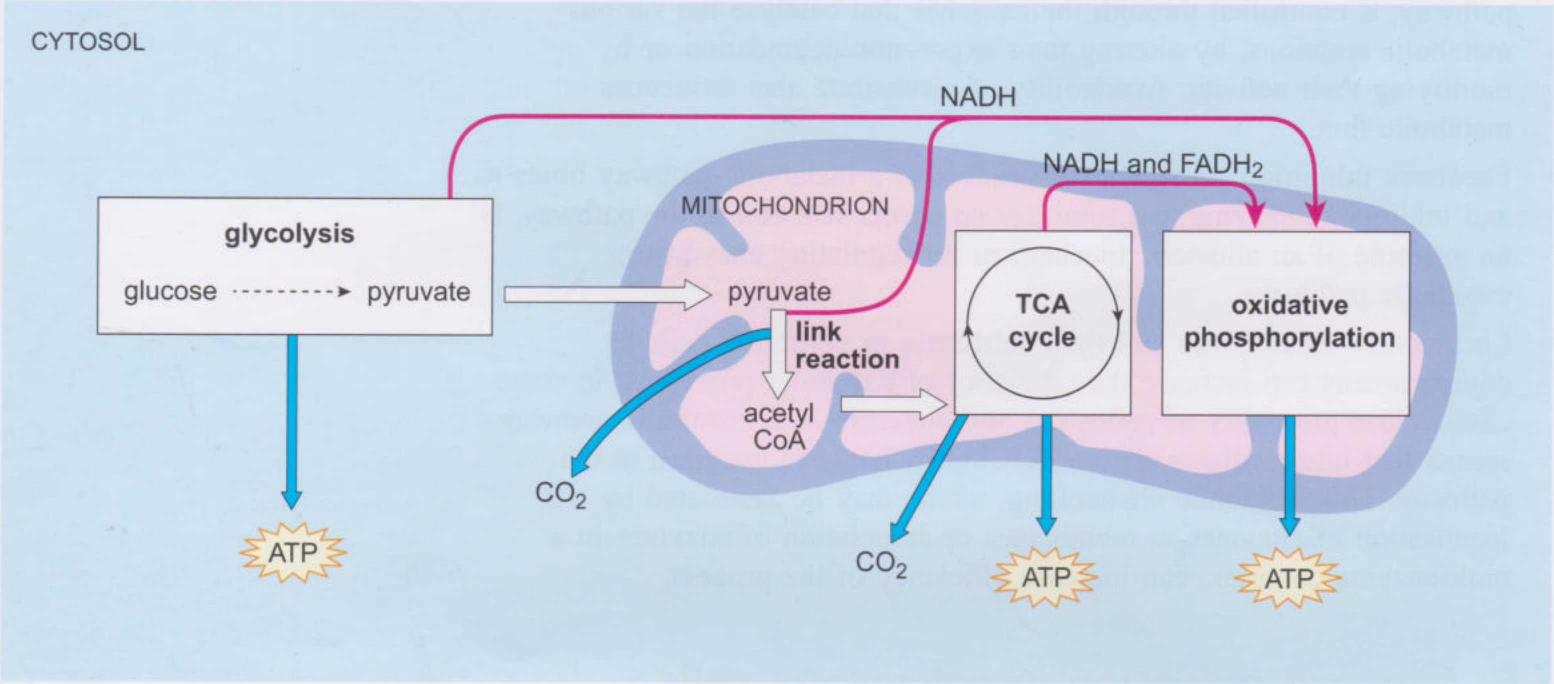


Figure 3.10 Overview of glucose oxidation (aerobic respiration). In glycolysis, each glucose molecule is broken down into two molecules of pyruvate. In eukaryotic cells, pyruvate enters the mitochondrion where it is broken down to form CO_2 and an acetyl group, which is transferred to acetyl CoA. This reaction is called the link reaction because it links glycolysis to the TCA cycle. Acetyl CoA enters the TCA cycle, which completes the oxidation process, producing more CO_2. In the process, the coenzymes NAD^+ and FAD are reduced to NADH and $FADH_2$, respectively, and ADP is phosphorylated to make ATP. During oxidative phosphorylation, chemical energy from the reduced coenzymes NADH and $FADH_2$ is converted into a form that can be used to synthesise ATP. The details of these processes are described in the text.

Unlike the direct combustion of glucose in air, which releases energy as heat, in aerobic respiration, the energy produced is almost entirely converted into useful chemical bond energy in the form of ATP, with a theoretical yield of 38 molecules of ATP for the oxidation of one molecule of glucose by aerobic respiration.

Oxidation of glucose in aerobic respiration is achieved in four stages:

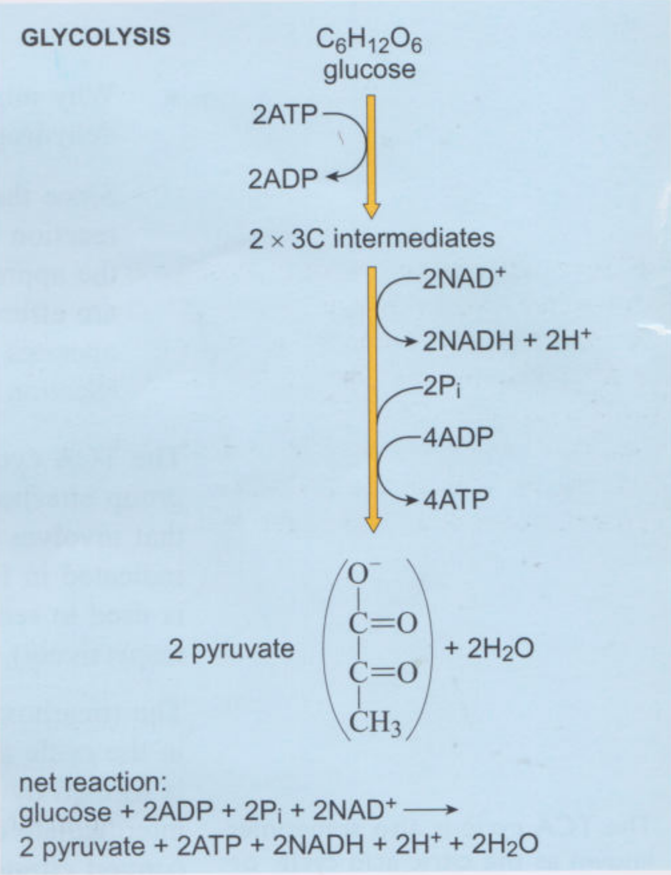
1 glycolysis
2 the link reaction
3 the tricarboxylic acid (TCA) cycle, and
4 oxidative phosphorylation.

These pathways/reactions are located in distinct compartments within the cell. Thus glycolysis occurs in the cytosol, the link reaction and the TCA cycle both occur in the mitochondrial matrix, and oxidative phosphorylation occurs at the inner mitochondrial membrane.

Though the details of the oxidation of other organic molecules will not be discussed here, at this stage it is worth noting that the breakdown of fatty acids, a major energy source in foods, generates large amounts of acetyl CoA, the product of the link reaction. Through this intermediate, energy from fatty acids can be channelled directly into the TCA cycle.

3.6.1 Glycolysis

Glycolysis is a series of ten reactions, each catalysed by a different enzyme. Collectively, these reactions convert a glucose molecule ($C_6H_{12}O_6$) containing six carbons into two pyruvate molecules, each containing three carbons. For every molecule of glucose entering glycolysis, two molecules of ATP are hydrolysed to ADP in order to provide energy for the early steps, and the phosphate groups from these ATP molecules are transferred to intermediates in the pathway (in phosphate transfer reactions, Section 3.4). There then follows a reaction in which a 6-carbon intermediate is cleaved to give two 3-carbon molecules. These molecules are further oxidised to give pyruvate, and the energy released is used to reduce NAD^+ to NADH and to phosphorylate ADP. Indeed, despite the initial investment of two ATP molecules, there is a net gain of two ATP and two NADH for each molecule of glucose that goes through glycolysis, as shown in Figure 3.11.



net reaction:
$$\text{glucose} + 2ADP + 2P_i + 2NAD^+ \longrightarrow$$
$$2\text{ pyruvate} + 2ATP + 2NADH + 2H^+ + 2H_2O$$

Figure 3.11 Overview of glycolysis, the first stage in the oxidation of glucose. Hydrolysis of two ATP molecules is required to drive the early reactions. There is net production of two ATP and two NADH for each molecule of glucose that goes through glycolysis. The overall reaction is indicated beneath the figure. The two pyruvate molecules produced by glycolysis enter the mitochondria where they are substrate for the link reaction.

3.6.2 The link reaction and TCA cycle

The next step in aerobic respiration is the **link reaction**, so called because it links glycolysis and the tricarboxylic acid (TCA) cycle. The link reaction (and the TCA cycle that follows) takes place in the mitochondrial matrix, so pyruvate formed by glycolysis in the cytosol has to pass through both the inner and outer membranes of the mitochondria before it can become a substrate for the link reaction.

■ The outer mitochondrial membrane is freely permeable to small carbon intermediates, but the inner membrane is not. Suggest how a charged intermediate like pyruvate might pass through the hydrophobic bilayer of the inner mitochondrial membrane.

□ By attaching to a specific, membrane-bound carrier protein (see Section 2.8.1).

The carrier that moves pyruvate across the inner mitochondrial membrane is known as the pyruvate transporter. The link reaction is actually a series of reactions catalysed by three different enzymes, which are assembled in a large multienzyme complex known as the **pyruvate dehydrogenase complex (PDC)**. The net result of the series of three reactions catalysed by the PDC is that pyruvate is cleaved to release CO_2, leaving a 2-carbon acetyl group that is transferred to coenzyme A, and, in the process, NAD^+ is reduced to NADH (Figure 3.12).

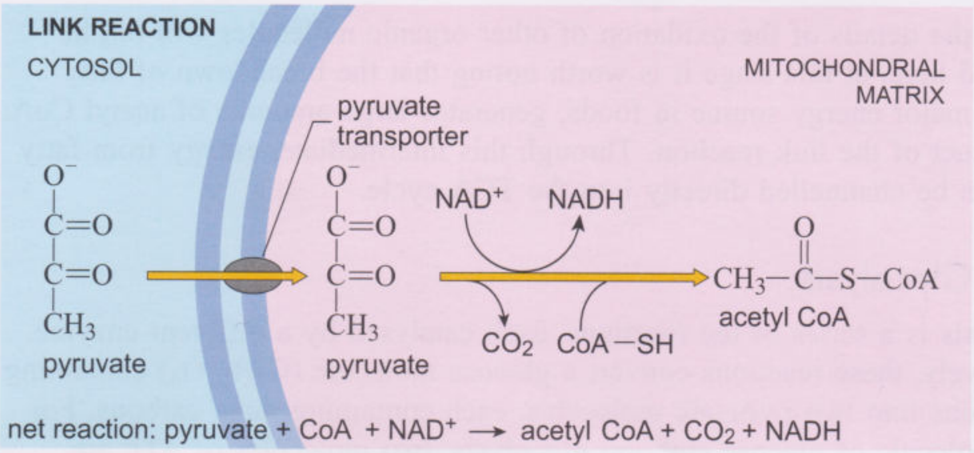


Figure 3.12 The link reaction is actually a series of three reactions catalysed by the three different enzymes that make up the pyruvate dehydrogenase complex (PDC). Together, these enzymes cleave a 3-carbon pyruvate molecule to release CO_2 and transfer a 2-carbon acetyl group to coenzyme A, reducing NAD^+ to NADH in the process. The overall reaction is indicated beneath the figure.

■ Why might the association of the three enzymes in the pyruvate dehydrogenase complex enhance the efficiency of the link reaction?

□ Since the enzymes are all in close physical proximity, the product of one reaction step, which is also a reactant in the next step, can rapidly pass to the appropriate enzyme in the complex. Thus the products and substrates are effectively concentrated in the complex and the enzyme complex operates more efficiently. This is an example of metabolic channelling (Section 3.5.4).

The TCA cycle completes the breakdown of glucose, by oxidising the acetyl group attached to coenzyme A to form CO_2. It does so in a cyclical pathway that involves eight reactions, each catalysed by a different enzyme. As indicated in Figure 3.13, the energy released on oxidation of the acetyl group is used to reduce the coenzymes NAD^+ and FAD (to NADH and $FADH_2$, respectively), and to phosphorylate ADP to make ATP.

The tricarboxylic acid cycle is so named because citrate, the first intermediate in the cycle after acetyl CoA, has three carboxylate groups ($-COO^-$). Citrate is formed by the transfer of the acetyl group from acetyl CoA to the 4-carbon intermediate oxaloacetate (OAA). The next seven reactions of the cycle convert citrate back into OAA, ready to take on another acetyl group from acetyl CoA. The whole cycle can be seen as a vehicle for drawing 2-carbon fragments in from the link reaction and releasing them as CO_2. The reduced coenzymes NADH and $FADH_2$ are the major, energy-carrying products of the TCA cycle. As you will see in the following section, they ultimately deliver this energy to the final step in oxidation of glucose, the electron transport chain.

The TCA cycle is also sometimes known as the citric acid cycle; or the Krebs cycle after Hans Krebs, the scientist principally responsible for elucidating the pathway in the 1930s.

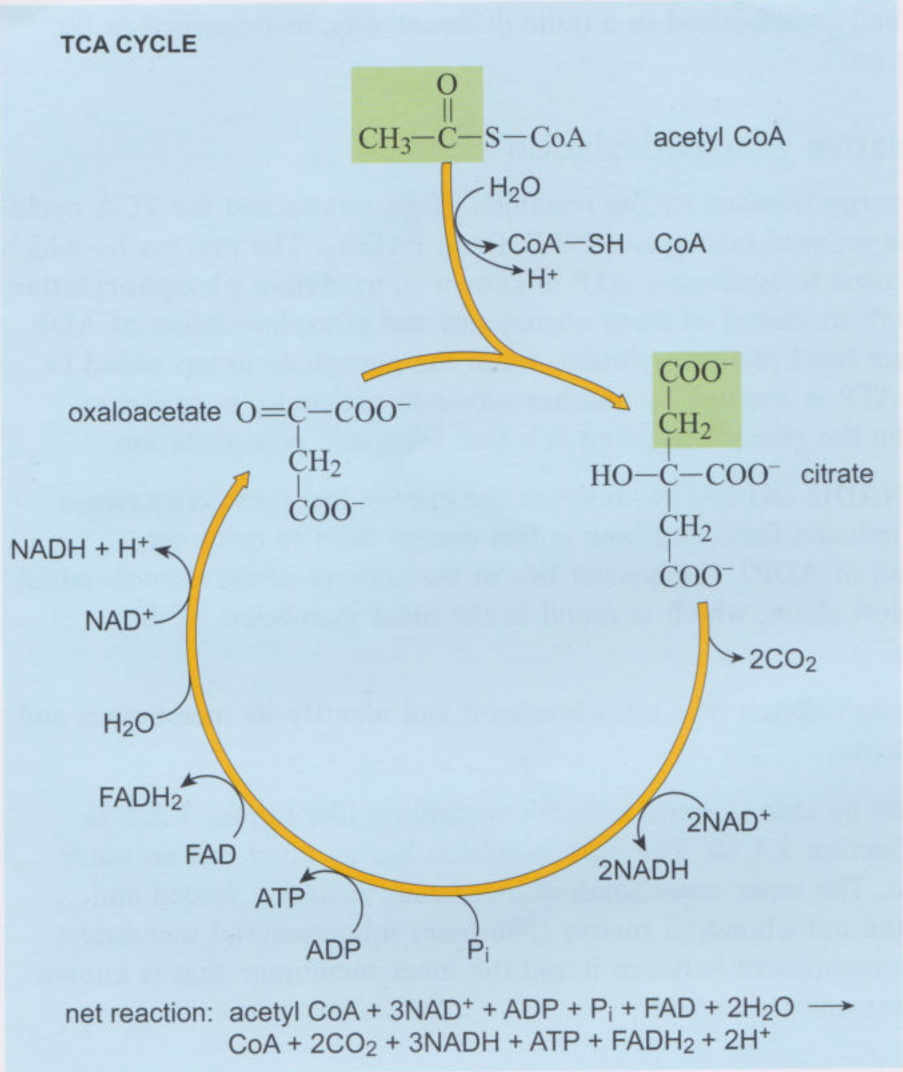

TCA CYCLE
$CH_3-C(=O)-S-CoA$
acetyl CoA
H_2O
CoA−SH CoA
H^+
oxaloacetate $O=C(-COO^-)-CH_2-COO^-$
$COO^- - CH_2 - C(OH)(COO^-) - CH_2 - COO^-$
citrate
$2CO_2$
2NAD+
2NADH
P_i
ADP
ATP
FAD
$FADH_2$
H_2O
NAD+
NADH + H+
net reaction: acetyl CoA + 3NAD+ + ADP + P_i + FAD + $2H_2O$ ⟶ CoA + $2CO_2$ + 3NADH + ATP + $FADH_2$ + 2H+

Figure 3.13 Overview of the TCA cycle showing the major inputs and outputs. For simplicity, the separate reactions of the cycle (of which there are eight) are not shown. The overall reaction is indicated beneath the figure. Since glycolysis yields two pyruvate molecules for every molecule of glucose, the TCA cycle turns twice for every glucose molecule oxidised. Hence oxidation of one glucose molecule yields six NADH, two $FADH_2$ and two ATP from the TCA cycle to add to those produced by glycolysis.

Without OAA, the link reaction cannot connect with the TCA cycle, and the whole of glucose oxidation would grind to a halt. This possibility is a very real danger in the cell, since OAA is an important intermediate that is often diverted to other biosynthetic reactions; so separate reactions that top up OAA levels from other sources are vital for energy metabolism. The TCA cycle is very efficient because, as well as generating energy-rich reduced coenzymes and ATP, it serves to produce organic intermediates for biosynthesis. This is particularly relevant in plants, where biosynthesis rather than energy production is the main role of the TCA cycle.

For every glucose molecule oxidised, there is a *net* production of only two ATP molecules by glycolysis and the same number from the TCA cycle. These four ATP molecules are produced by what is known as **substrate level phosphorylation**, in which a phosphate group is transferred from a substrate molecule to ADP. However, most of the ATP that is made in the oxidation of glucose is actually synthesised in a quite different way, as described in the following section.

3.6.3 Oxidative phosphorylation

Most of the energy released by the reactions of glycolysis and the TCA cycle is stored in the reduced coenzymes NADH and $FADH_2$. The process by which this energy is used to synthesise ATP is known as **oxidative phosphorylation** as it entails both oxidation of these coenzymes and phosphorylation of ADP. Unlike substrate level phosphorylation, when the phosphate group added to ADP to form ATP is donated by another substrate molecule, in oxidative phosphorylation the phosphate group is a free inorganic phosphate ion.

Oxidation of NADH and $FADH_2$ releases the energy that these coenzymes carry in their reduced form, but how is this energy used to drive the phosphorylation of ADP? The answer lies in the activity of the mitochondrial electron transport chain, which is found in the inner membrane of the mitochondrion.

- ■ Draw a rough sketch of a mitochondrion and identify its membranes and compartments.
- □ You should be able to draw a sketch something like Figure 3.22b in Book 1, Section 3.4.10. The mitochondrion has an inner and an outer membrane. The inner mitochondrial membrane is highly folded and encloses the mitochondrial matrix. The outer mitochondrial membrane forms a compartment between it and the inner membrane that is known as the intermembrane space.

The mitochondrial electron transport chain

The electrons that NADH and $FADH_2$ lose on oxidation are ultimately used to reduce molecular oxygen (O_2) to form water (H_2O). Oxygen is known as the terminal electron acceptor, and this is the reason that oxygen is so essential to aerobic life forms. Without oxygen, efficient respiration cannot occur. The high-energy electrons of NADH and $FADH_2$ do not pass directly to O_2, however, but pass through a series of protein complexes which act as electron carriers, and which are arranged in the inner mitochondrial membrane; hence the name mitochondrial **electron transport chain (ETC**; Figure 3.14).

Each of the electron carriers in the chain has a higher affinity (i.e. attraction) for electrons than the previous one. Thus in a sequence of linked redox reactions, electrons are passed from one electron carrier to the next in the series, represented in Figure 3.14. Each of the four large protein complexes of the ETC (numbered I to IV), referred to as **respiratory complexes**, consists of several proteins with associated prosthetic groups (Section 1.8.3). Electrons are transferred from NADH to complex I (also known as the NADH dehydrogenase complex) and passed to a highly mobile carrier called ubiquinone (Q in Figure 3.14). Ubiquinone in turn passes electrons to complex III (also known as the cytochrome *b*/*c1* complex). Cytochrome *c* shuttles back and forth, delivering electrons from complex III to complex IV (the cytochrome oxidase complex), which ultimately uses the electrons to

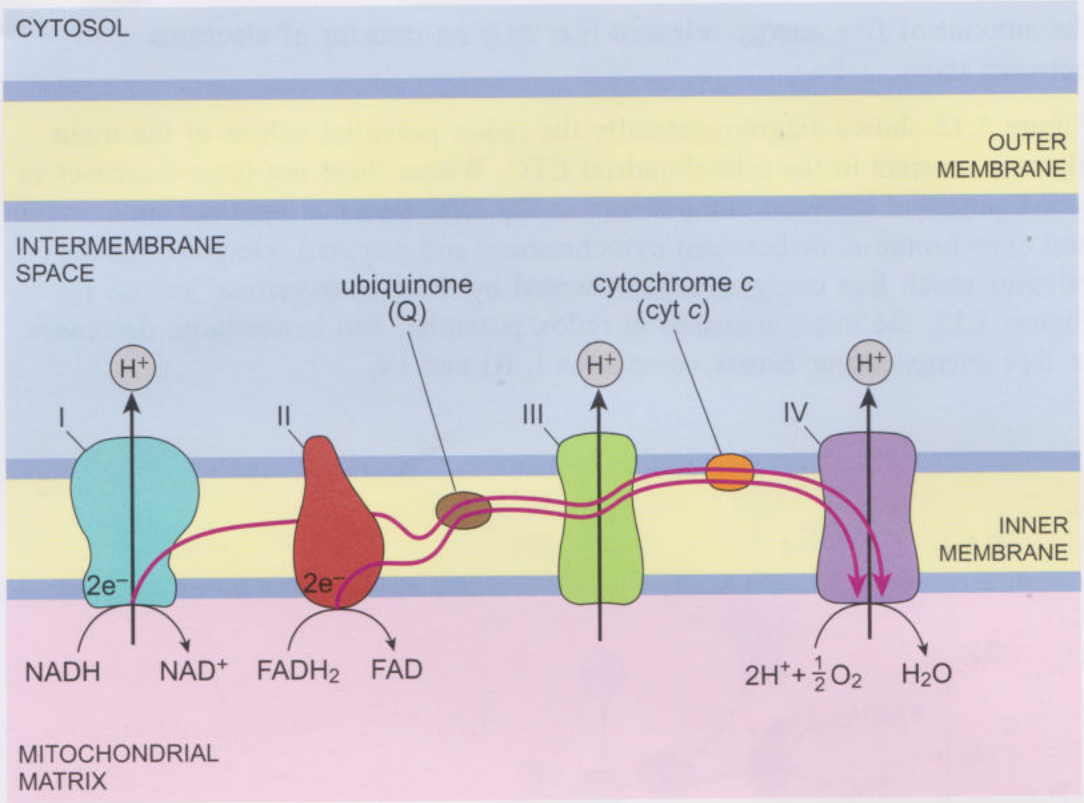


Figure 3.14 Components of the electron transport chain depicted *in situ* in the inner mitochondrial membrane. The chain consists of four protein complexes (numbered I–IV) and two mobile electron carriers, ubiquinone (Q) and cytochrome *c* (cyt *c*). The flow of electrons (e^-) along the chain is indicated by the pink arrows. Electrons are passed from either complex I or II (depending on whether they derive from the oxidation of NADH or $FADH_2$) to complex III and then to complex IV. For detailed explanation, see text.

reduce O_2. Oxidation of $FADH_2$ is catalysed by complex II and the electrons are passed to ubiquinone, from whence they are transferred through the ETC in the same way as those removed from NADH.

The passage of electrons through the ETC always occurs in the same direction, from NADH/$FADH_2$ to O_2, because each electron carrier of the ETC has a higher affinity for electrons than does the preceding carrier. The redox potential of each of the components of the ETC is a measure of its affinity for electrons, effectively its potential to be reduced or oxidised (See Box 3.1). Whether or not a particular electron carrier can accept electrons from its neighbour depends upon its redox potential. In mitochondria, for example, ubiquinone has a higher redox potential than carriers in complexes I and II, but a lower redox potential than those in complex III. It therefore readily accepts electrons from complex I or complex II and passes them on to complex III.

The point to remember is that low redox potential indicates low electron affinity, and electrons flow towards molecules with high redox potential. Recall that the transfer of electrons from a reactant with a low redox potential to one with higher redox potential is exergonic and, as stated in Box 3.1, the difference in redox potential between two compounds (ΔE) is proportional to the amount of free energy released (i.e. ΔG) on transfer of electrons between them.

Figure 3.15 shows diagrammatically the redox potential values of the main electron carriers in the mitochondrial ETC. Where there are large increases in redox potential between components in the ETC (such as between ubiquinone and cytochrome *c*, or between cytochrome *c* and oxygen), electron transfer releases much free energy. As represented by three near-vertical arrows in Figure 3.15, the large increases in redox potential, and hence large decreases in free energy, occur across complexes I, III and IV.

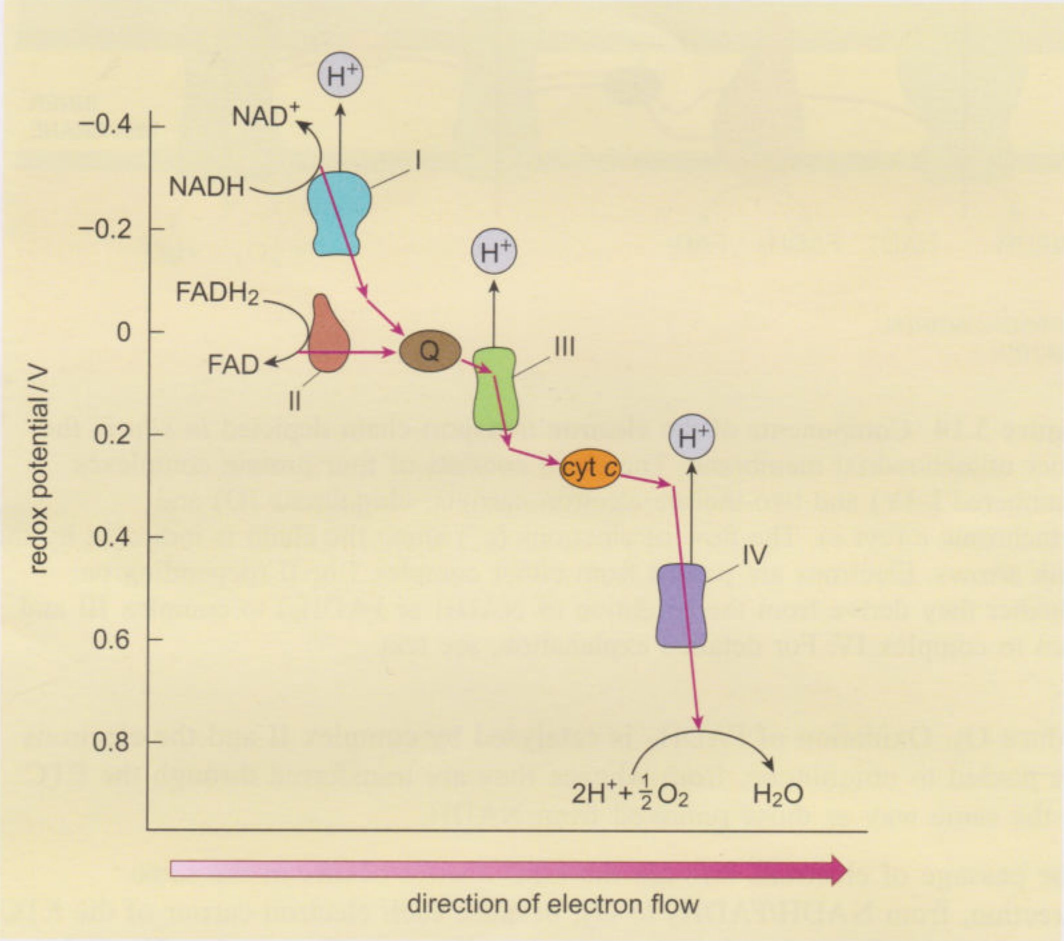


Figure 3.15 Redox potential of the electron carriers of the mitochondrial electron transport chain. The electron carriers are positioned according to their redox potential on the vertical axis. The difference in the redox potential between different components of the ETC dictates the flow of electrons through the chain, from compounds with low redox potentials to those with higher redox potentials.

The energy released from electron transfer is used to pump protons (H^+) across the membrane from the mitochondrial matrix into the space between the inner and outer mitochondrial membranes (the intermembrane space). This pumping of protons into the intermembrane space occurs at complexes I, III and IV (Figure 3.14), the sites at which sufficient energy is available, and is caused by allosteric changes in the conformation of the complex I, III and IV proteins. Proton pumping by these components of the electron transport chain generates a proton concentration gradient across the inner mitochondrial membrane as well as a potential difference (membrane potential) due to the build-up of a high positive charge in the intermembrane space relative to the matrix.

- ■ Recalling from the previous chapter, what does a combination of an ion concentration difference and a membrane potential across a membrane signify?
- □ It is an electrochemical gradient (Sections 2.7 and 2.8.3).

Energy is therefore needed to pump protons into the intermembrane space against this **electrochemical proton gradient**. The gradient is such that the intermembrane space becomes markedly more acidic than the matrix (a difference of 1.4 pH units). The free energy stored in this electrochemical proton gradient is known as the **proton motive force** and, as you will see in the following section, it is this that drives synthesis of ATP.

- ■ Comment on the relative proton motive force generated by oxidation of $FADH_2$ and NADH in the ETC.
- □ Electrons from $FADH_2$ pass via complex II to ubiquinone, and then to complexes III and IV; whereas electrons from NADH pass through complexes I, III and IV. Since electron transfer through complexes I, III and IV contributes to generation of the proton motive force, it follows that the proton motive force generated by oxidation of $FADH_2$ is less than that generated by NADH.

The chemiosmotic coupling model

ATP synthesis in oxidative phosphorylation is catalysed by a large enzyme, called **ATP synthase**, which, like the components of the electron transport chain, is situated in the inner mitochondrial membrane. In fact, this enzyme constitutes about 15% of the total protein content of the inner mitochondrial membrane.

ATP synthase offers a route for the protons that have accumulated in the intermembrane space to flow down their electrochemical gradient across the inner membrane and back into the mitochondrial matrix. As protons pass through ATP synthase, the energy that was stored in the proton gradient is released and used to drive the endergonic synthesis of ATP from ADP and inorganic phosphate (P_i) (Figure 3.16). Thus the electrochemical proton gradient *couples* electron transfer along the electron transport chain to ATP synthesis. This coupling of electron transport and ATP synthesis by means of an electrochemical gradient is known as **chemiosmotic coupling** and was described by Peter Mitchell in 1961. Mitchell was awarded the Nobel Prize in 1978 for his ground-breaking work in this area.

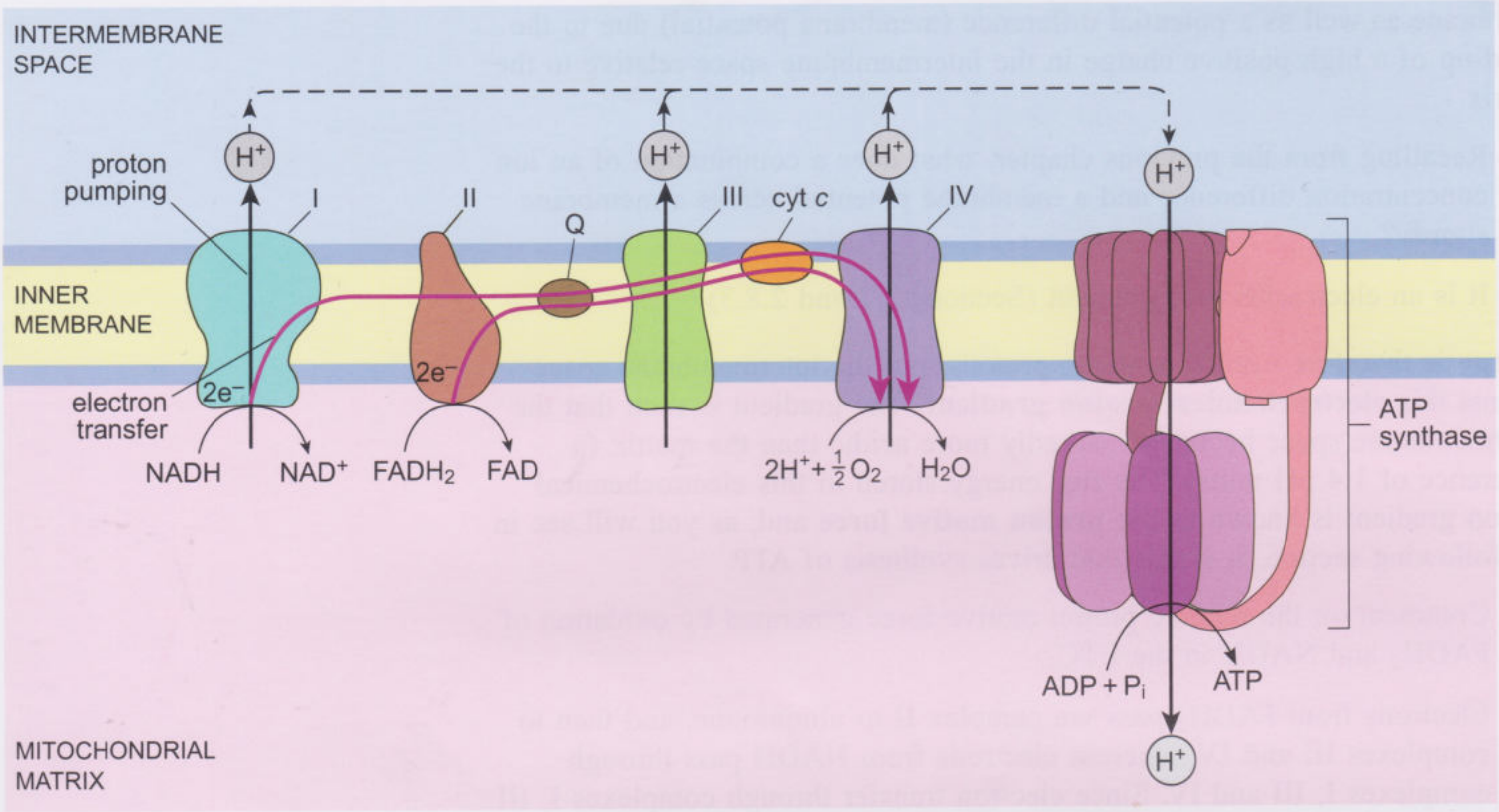


Figure 3.16 Chemiosmotic coupling of the electron transport chain to ATP synthesis in oxidative phosphorylation. See text for details.

ATP synthase is a large multimeric protein with a highly complex structure which, at the time of writing (spring 2012), is yet to be fully determined. ATP synthase in the mitochondria of animal cells consists of 15 different types of subunit, with multiples of many of these. From a mechanistic point of view, the ATP synthase complex can be considered as consisting of four major components: a rotor, an internal rod, a catalytic head and a stationary part called a stator; as depicted in Figure 3.17.

Protons (H^+) from the intermembrane space enter a channel in the stator which directs them into binding sites on the rotor, causing it to spin within the membrane. As the rotor spins, protons are discharged into the mitochondrial matrix and the internal rod that extends into the catalytic head spins too. The stator holds the catalytic head stationary while the turning of the internal rod activates its catalytic activity and ATP is synthesised from ADP and P_i. It is calculated that approximately 2.7 protons pass through ATP synthase for every molecule of ATP synthesised. Thus ATP synthase is effectively a rotary motor that is driven by protons. It is the smallest such molecular motor known in nature. Remarkably, during ATP synthesis, the central rotor turns about 150 times per second!

Note that while respiring and photosynthetic organisms use ATP synthase for ATP synthesis, in organisms living on fermentation, ATP synthase works in reverse to that described here. In such cases it hydrolyses ATP and generates an electrochemical proton gradient across a membrane. In *E. coli*, both ATP synthesis and ATP-driven proton pumping functions are important, depending on the cell environment. Thus, under anaerobic conditions, ATP synthase in

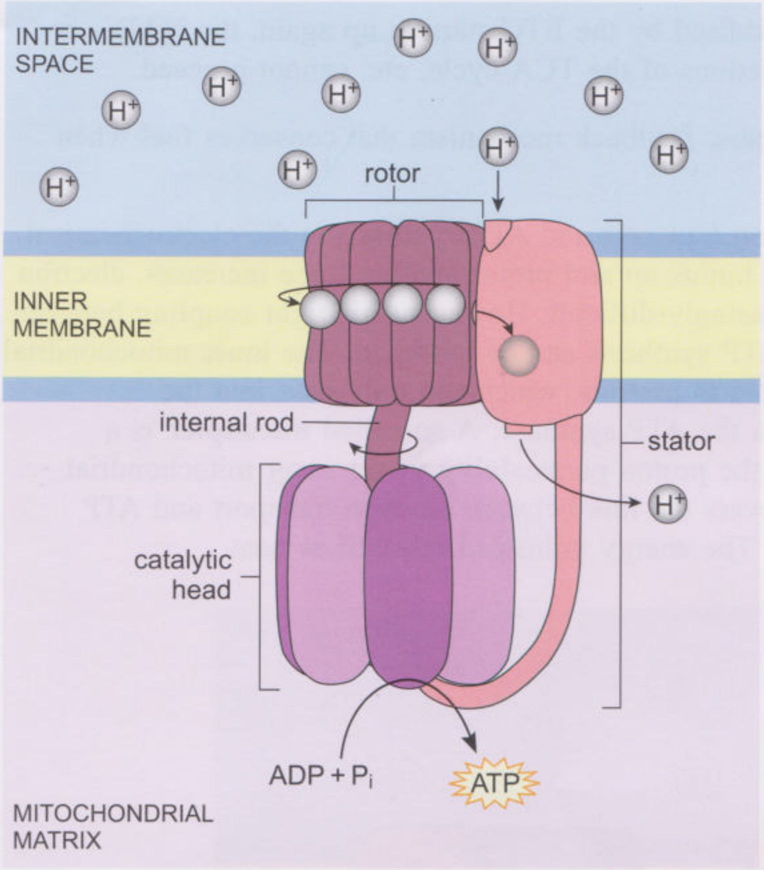


Figure 3.17 Structure of ATP synthase. This large multimeric protein has four components – a rotor, an internal rod, a stator and a catalytic head. The mechanism by which ATP synthase uses the electrochemical proton gradient to make ATP is described in the text. Note that for historical reasons, the soluble globular part that extends into the matrix is known as F_1, and the membrane-bound part is known as F_0; thus the protein is sometimes described as the F_0–F_1 synthase.

E. coli can act as an ATP-driven proton pump, generating a proton gradient that can power membrane transporters and flagellar movement.

Respiratory control

The rate at which an organism oxidises its fuel supply is carefully linked to its energy needs. Some of the mechanisms by which metabolic processes are controlled were described in Section 3.5. However, in mitochondria, there is a further extremely important control point – the coupling of the electron transport chain to ATP synthesis. The activity of the electron carriers depends on the activity of ATP synthase; when ATP synthesis slows down because ATP supplies are fully topped up, then the electron carriers slow down too. This phenomenon is known as **respiratory control**. It is very beneficial in the energy economy of the cell.

■ How would the coupling of electron transport to ATP synthesis prevent the wasteful breakdown of fuel compounds?

□ When ATP needs are satisfied and ATP synthase activity slows down, good respiratory control ensures that electron transport also slows, which leads to an accumulation of reduced coenzymes (NADH and $FADH_2$).

> Until these are reoxidised by the ETC starting up again, the NAD^+- and FAD-dependent reactions of the TCA cycle, etc. cannot proceed.

Hence there is an automatic feedback mechanism that conserves fuel when ATP is plentiful.

The link between electron transport and ATP synthesis is the electrochemical proton gradient. As this builds up and proton motive force increases, electron transport becomes increasingly difficult. However, this tight coupling between electron transport and ATP synthesis can be loosened. The inner mitochondrial membrane is impermeable to protons, which can only pass into the mitochondrial matrix via the ATP synthase. A so-called **uncoupler** is a molecule that increases the proton permeability of the inner mitochondrial membrane and hence severs the link between electron transport and ATP synthesis (Figure 3.18). The energy is instead released as heat.

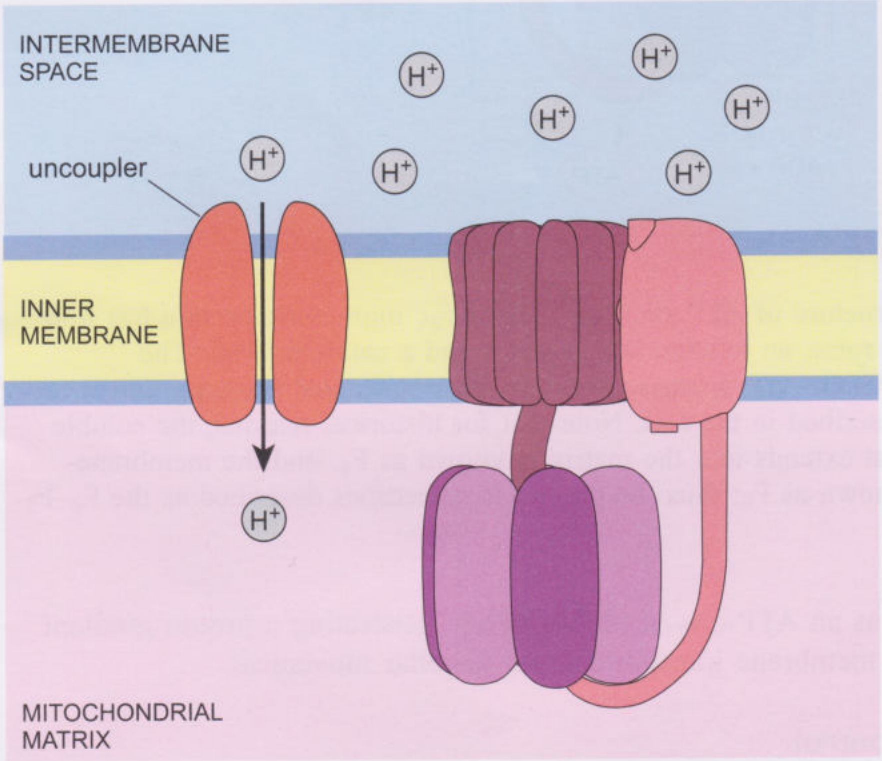


Figure 3.18 Uncouplers provide alternative, non-ATP-generating routes for protons returning to the mitochondrial matrix. The uncoupler found in brown adipose tissue in mammals forms channels in the inner mitochondrial membrane, through which protons can pass (as represented here). Other, exogenous uncouplers ferry protons across the inner mitochondrial membrane, thereby uncoupling ATP synthesis from electron transport.

The inner membrane of the many mitochondria in brown adipose tissue in mammals contains an uncoupler protein that forms an alternative proton channel. This uncoupling protein is central to the function of brown adipose tissue as a specialised type of thermogenic (heat-generating) tissue in mammals.

Protons returning to the mitochondrial matrix via the uncoupler rather than through the ATP synthase do not contribute energy for ATP synthesis. Thus the proton gradient is dissipated without making ATP, and the energy is released as heat instead. The H^+ channel formed by the uncoupler in brown adipose tissue is subject to regulation, so the generation of heat is controlled. Brown adipose tissue is important for the survival of small mammals in a cold environment and in generation of heat during arousal from hibernation.

Certain toxic compounds, such as 2,4-dinitrophenol and related compounds, previously marketed as 'slimming' drugs but now little used, can also act as uncouplers. Only very small amounts are needed to have an effect, because each uncoupler molecule is regenerated. One uncoupler molecule picks up a proton from the intermembrane space and moves through the membrane with it, depositing it in the matrix. It then returns through the membrane for another proton.

3.6.4 How much ATP is produced from aerobic respiration?

This section considers aerobic respiration as a whole. The net reactions for glycolysis, the link reaction and the TCA cycle are summarised below.

Glycolysis:

$$\text{glucose} + 2\text{ADP} + 2\text{P}_i + 2\text{NAD}^+ \rightarrow 2\text{ pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}$$

Link reaction:

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

TCA cycle:

$$\text{acetyl CoA} + 3\text{NAD}^+ + \text{ADP} + \text{P}_i + \text{FAD} + 2\text{H}_2\text{O} \rightarrow \text{CoA} + 2\text{CO}_2 + 3\text{NADH} + \text{ATP} + \text{FADH}_2 + 2\text{H}^+$$

■ Calculate the total yield of ATP, NADH and $FADH_2$ obtained from the complete oxidation of one molecule of glucose.

□ Remembering that two molecules of pyruvate are produced from one molecule of glucose as a result of glycolysis, the total yields of these energy carriers are as summarised in Table 3.3.

To calculate the overall yield of ATP per molecule of glucose oxidised, you will also need to determine how many ATPs are synthesised for each NADH or $FADH_2$ that is oxidised in oxidative phosphorylation. Oxidation of NADH, as you learnt in Section 3.6.3, generates more proton motive force than does $FADH_2$. The electrochemical proton gradient generated when NADH passes its high-energy electrons to O_2 via the ETC is theoretically sufficient to drive the synthesis of three ATP whilst each $FADH_2$ oxidised by the ETC theoretically yields two ATP.

Table 3.3 Yield of ATP and reduced coenzymes from oxidation of one molecule of glucose.

Pathway in aerobic respiration	**ATP**	**NADH**	**$FADH_2$**
Glycolysis	2	2	
Link reaction		2	
TCA cycle	2	6	2
Total	**4**	**10**	**2**

Note: Glycolysis of one glucose molecule produces four ATP, but uses two ATP; and each of the two acetyl CoA molecules generates one ATP in the TCA cycle – a total net gain of four ATP via substrate level phosphorylation.

- ■ From the information supplied above, calculate the maximum theoretical yield of ATP molecules produced as a result of oxidation of one molecule of glucose, assuming all the reduced coenzymes are oxidised by the ETC.

- □ Oxidative phosphorylation would theoretically yield 30 ATP from oxidation of the 10 NADH and four ATP from oxidation of two $FADH_2$. Together with the four ATP produced by substrate level phosphorylation in glycolysis and the TCA cycle, this gives a theoretical maximum yield of 38 ATP molecules per molecule of glucose oxidised by aerobic respiration.

In practice, the yield of ATP from oxidation of glucose by aerobic respiration is less than this theoretical maximum. The reason is that energy is expended to transport NADH produced by glycolysis from the cytosol into the mitochondrial matrix. Similarly, pyruvate, ADP and P_i must also be transported into the mitochondrion. As a result, the yield of ATP is actually about 30 molecules per molecule of glucose oxidised.

3.6.5 Glucose oxidation in anaerobic conditions

As you have seen in the previous section, the majority of the ATP produced in aerobic respiration is derived from oxidative phosphorylation. In anaerobic organisms, i.e. those that do not use or therefore need O_2 (e.g. certain yeasts), and in certain animal tissues such as skeletal muscle when O_2 supply becomes limiting during vigorous exercise, glycolysis is the principal source of ATP.

- ■ What is the yield of ATP per glucose in anaerobic conditions?

- □ Two ATP per glucose would be produced by glycolysis.

- ■ What would happen to glycolysis if there was no mechanism to reoxidise NADH to NAD^+?

- □ Glycolysis would grind to a halt because it requires NAD^+.

So, under anaerobic conditions, in the absence of oxidative phosphorylation, the NADH produced by glycolysis must be reoxidised by another means; otherwise the cell would quickly run out of NAD^+.

Normally in mammalian tissues, the product of glycolysis, pyruvate, passes into the mitochondrial matrix and is converted into acetyl CoA in the link reaction. However, in O_2-limited conditions in skeletal muscle, the pyruvate and NADH produced by glycolysis remain in the cytosol and NADH is used to reduce pyruvate to lactate, thereby regenerating NAD^+ which is required for continued glycolysis (Figure 3.19a). In yeast growing in anaerobic conditions, pyruvate is converted into ethanol and CO_2, again using the reducing power of NADH, and the resulting NAD^+ feeds back into glycolysis (Figure 3.19b). Pathways such as these, in which the electrons (or reducing power) extracted from the nutrient (glucose) are transferred to the product of breakdown of the nutrient (pyruvate) are known as **fermentations**.

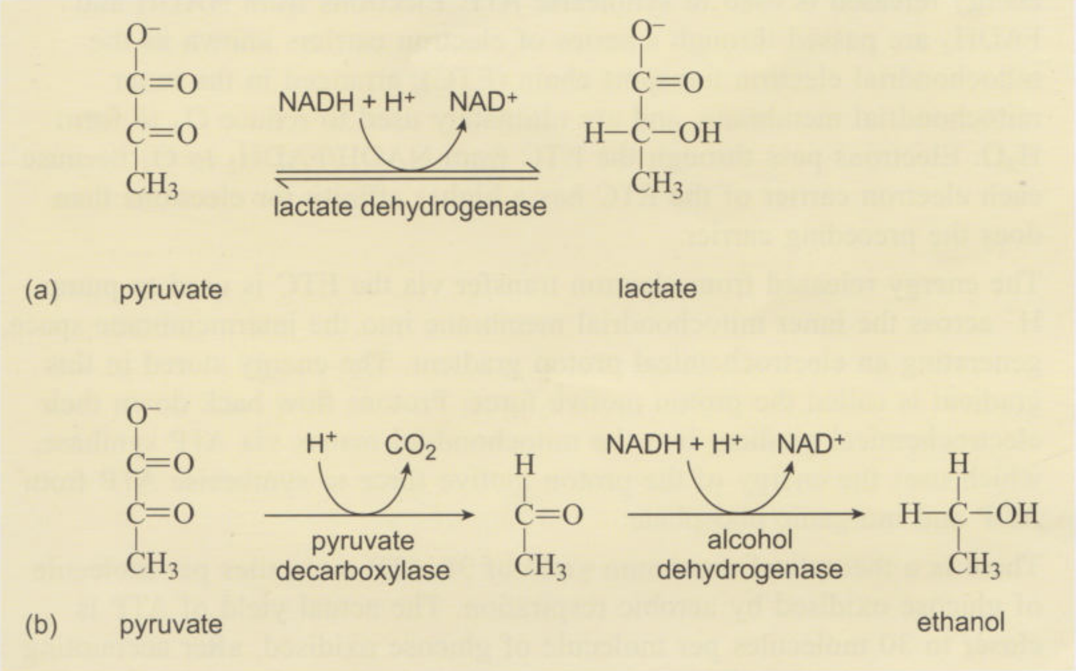


Figure 3.19 (a) Anaerobic breakdown of pyruvate to lactate such as occurs in vigorously contracting skeletal muscle cells in which O_2 is limited. (b) Anaerobic breakdown of pyruvate to CO_2 and ethanol, as occurs in yeasts.

Summary of Section 3.6

- Aerobic respiration is the oxidation of glucose by the cell in the presence of oxygen.
- Oxidation of glucose in aerobic respiration is achieved in four stages: glycolysis in the cytosol; the link reaction and the TCA cycle in the mitochondrial matrix; and oxidative phosphorylation at the inner mitochondrial membrane.
- Glycolysis is a series of ten reactions that convert a glucose molecule ($C_6H_{12}O_6$) containing six carbons into two pyruvate molecules, each containing three carbons. There is a net gain of two ATP (as a result of substrate level phosphorylation) and two NADH for each molecule of glucose that goes through glycolysis.
- Pyruvate formed by glycolysis is transported from the cytosol into the mitochondrial matrix where it can become a substrate in the link reaction catalysed by the pyruvate dehydrogenase complex. The pyruvate is cleaved

to generate CO_2 and a 2-carbon acetyl group that is transferred to coenzyme A, and, in the process, NAD^+ is reduced to NADH.

- The TCA cycle completes the breakdown of glucose by oxidising the acetyl group attached to coenzyme A to form CO_2. The energy released on oxidation of the acetyl group is used to reduce the coenzymes NAD^+ and FAD, and to phosphorylate ADP (by substrate level phosphorylation) to make ATP.
- In oxidative phosphorylation, NADH and $FADH_2$ produced during glycolysis, the link reaction and the TCA cycle are oxidised and the energy released is used to synthesise ATP. Electrons from NADH and $FADH_2$ are passed through a series of electron carriers known as the mitochondrial electron transport chain (ETC), arranged in the inner mitochondrial membrane, and are ultimately used to reduce O_2 to form H_2O. Electrons pass through the ETC from NADH/$FADH_2$ to O_2 because each electron carrier of the ETC has a higher affinity for electrons than does the preceding carrier.
- The energy released from electron transfer via the ETC is used to pump H^+ across the inner mitochondrial membrane into the intermembrane space, generating an electrochemical proton gradient. The energy stored in this gradient is called the proton motive force. Protons flow back down their electrochemical gradient into the mitochondrial matrix via ATP synthase, which uses the energy of the proton motive force to synthesise ATP from ADP and inorganic phosphate.
- There is a theoretical maximum yield of 38 ATP molecules per molecule of glucose oxidised by aerobic respiration. The actual yield of ATP is closer to 30 molecules per molecule of glucose oxidised, after accounting for energy expended during transport of intermediates between cellular compartments.
- In anaerobic organisms, and in certain animal tissues such as skeletal muscle when O_2 supply becomes limiting, glycolysis is the principal source of ATP. In such cases, fermentation pathways use NADH produced by glycolysis to reduce pyruvate, regenerating NAD^+. In yeasts, for example, the products of fermentation are CO_2 and ethanol.

3.7 Oxidative phosphorylation in prokaryotes

The strategy of using an electron transport chain to generate energy for making ATP is seen in many different life forms. The underlying principles are the same, but the electron donors and carriers involved differ.

3.7.1 Chemoheterotrophic bacteria

All chemoheterotrophs obtain energy by oxidising organic compounds, but bacteria, depending on their genetic make-up, demonstrate a much greater diversity than animals in the organic molecules that they can metabolise; i.e. not just carbohydrate, fat and protein. Collectively, the prokaryotes are able to metabolise virtually any organic compound. For example, some species have the necessary enzymes for growing on organic acids, on phenols (the acidic components of coal tar) or on many other compounds. Most of these bacteria produce NADH by feeding their particular nutrient into the appropriate point in glycolysis, the link reaction or the TCA cycle. However, some reduce other coenzymes such as FAD.

Prokaryotes do not, as you learnt in Chapters 2 and 3 of Book 1, have mitochondria, and the components of the bacterial ETC are located in the cell membrane.

■ Where would protons be pumped during electron transfer in the ETC of Gram-positive and Gram-negative bacteria (described in Section 3.3 of Book 1)?

□ In Gram-positive bacteria, the protons would be pumped into the space between the plasma membrane and the bacterial cell wall; in Gram-negative bacteria, they would be pumped into the space between the plasma membrane and the outer membrane. In both Gram-positive and Gram-negative, these spaces are termed the periplasmic space (Figure 3.6 in Book 1).

Bacterial ETCs are much more variable than those of mitochondria, which are virtually the same in all eukaryotes. The structures of the electron carriers in bacterial ETCs reflect the need for different redox potentials when accepting electrons from different sources. Indeed this variability underpins bacterial diversity, allowing different species to colonise environments providing different nutrients, and still make ATP by chemiosmotic coupling.

Some bacteria continue to make ATP in this way even in the absence of oxygen. They are the anaerobic heterotrophs that can use alternatives to oxygen as the terminal electron acceptor. For example, the denitrifying bacteria (prominent in the nitrogen cycle) pass electrons to nitrate ions (NO_3^-) rather than oxygen, while some sulfate reducers (important in the sulfur cycle) pass electrons to sulfate ions (SO_4^{2-}) instead of oxygen.

The nitrogen and sulfur cycles are examples of biogeochemical cycles by which chemical elements are constantly recycled through the different compartments of Earth (organisms, soil, water and atmosphere).

■ In terms of ATP yield, why is using alternative terminal electron acceptors a better way of dealing with anaerobic conditions than the fermentation pathways (Section 3.6.5)?

□ Fermentation pathways only generate ATP from substrate level phosphorylation in glycolysis. The NADH that is produced by glycolysis cannot be reoxidised by the ETC because of the anaerobic conditions, and is instead reoxidised to NAD^+ without making ATP. Thus much less ATP is produced by fermentation pathways than by oxidative phosphorylation with an alternative to oxygen as the terminal electron acceptor.

3.7.2 Chemoautotrophs

Certain very interesting Bacteria and Archaea have specialised electron transport chains that allow them to accept electrons from inorganic compounds. They still make ATP by oxidative phosphorylation, but they have no need for organic molecules to provide reduced coenzymes to feed into the ETC. Indeed, some remarkable chemoautotrophs can grow on just an inorganic salt solution, with CO_2 as their sole carbon source. Inorganic electron sources may include iron (Fe^{2+}) and sulfide (S^{2-}) ions as well as ammonia (NH_3), H_2 or even carbon monoxide (CO) gas.

Several chemoautotrophs are economically and environmentally important. By oxidising nitrogen or sulfur compounds, some play a big part in the nitrogen and sulfur cycles. Others oxidise metal ions such as Fe^{2+}, and so can detoxify mining waste and recover useful metals as soluble salts. Another useful group is the methanogens, anaerobic Archaea that generate methane by reduction of CO_2 by H_2 gas:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

Methanogens (discussed further in Book 3, Chapter 2) also generate methane 'wind' in the gut of humans and other animals, especially ruminants.

When using these various inorganic sources of electrons, specialised electron carriers are needed for the first oxidation step. These proteins pass the electrons on to an ETC similar to that in mitochondria, although there are usually fewer than three proton pumping sites. Hence in chemoautotrophs, ATP yields (per pair of electrons) tend to be lower than in mitochondria. Exceptions are those using H_2 or CO as the inorganic electron source; their strongly negative redox potentials mean there is a large free energy drop when electrons are passed from them, via the ETC, to oxygen as the terminal electron acceptor.

Anaerobic chemoautotrophs that do not use oxygen as their terminal electron acceptor also have low ATP yields because the various alternative acceptors (e.g. nitrate) have less positive redox potential values than oxygen. Hence the total increase in redox potential during electron transport is lower and less energy is released. However, despite these disadvantages, chemoautotrophs are an important group, able to produce ATP by oxidative phosphorylation under unusual conditions.

3.7.3 Where did oxidative phosphorylation begin?

Making ATP is so basic to life it is hardly surprising that the high-yield chemiosmotic process (oxidative phosphorylation) is used by both eukaryotes and prokaryotes. The prokaryotes evolved first, of course, and most people now accept that mitochondria evolved from them as a result of endosymbiosis (Book 1, Section 1.2.4). Indeed the ETC components of *Paracoccus* are so similar to those of mitochondria, that this heterotrophic bacterium has been likened to a free-living mitochondrion. It could share a common ancestor with the bacterium from which all of today's mitochondria have evolved, the original bacterium having established a symbiotic relationship with another cell from which primitive eukaryotic organisms evolved.

Summary of Section 3.7

- Bacterial ETCs are much more variable than those of mitochondria, which are virtually the same in all eukaryotes. The structures of the electron carriers reflect the need for different redox potentials when accepting electrons from different sources.

- Anaerobic heterotrophs use alternatives to oxygen as the terminal electron acceptor; e.g. denitrifying bacteria pass electrons to nitrate rather than oxygen, sulfate reducers pass electrons to sulfate ions.
- Chemoautotrophic Bacteria and Archaea that use inorganic ions or compounds (e.g. Fe^{2+}, S^{2-}, NH_3, H_2, CO) as a source of energy have ETCs that allow them to accept electrons from these inorganic chemicals, but they still make ATP by oxidative phosphorylation.

3.8 Using light energy to make ATP

Plants and microbes with light-absorbing pigments can synthesise ATP by a rather different membrane-based mechanism called **photophosphorylation**. The simplest organism that uses light to make ATP is the purple-membrane archaeon, *Halobacterium halobium*. Purple patches in its cell membrane contain a molecular complex that includes a light-absorbing pigment (retinal) and a proton pump (bacteriorhodopsin, Figure 2.13). The energy of absorbed light is used to power structural rearrangements within the complex, and as a result, protons are pumped into the periplasmic space between the cell membrane and the outer wall of *H. halobium*, forming a transmembrane proton gradient. ATP is then made as protons flow back into the cytoplasm, through a membrane-bound ATP synthase.

This simple system has a major problem: *H. halobium* cannot synthesise ATP in this way in the dark. In fact, *H. halobium* only makes purple patches and photophosphorylates when oxygen levels are low, as a survival mechanism in adverse conditions; otherwise, this archaeon uses oxidative phosphorylation to make ATP.

More sophisticated photophosphorylating organisms can also carry out **photosynthesis**, storing the light energy available during the day as carbohydrate, which can be metabolised or oxidised to release energy, whether or not the Sun is shining. Photosynthesis can be simply represented by the left-to-right reaction below:

$$\underset{\text{carbon dioxide}}{n\mathrm{CO_2}} + \underset{\text{water}}{n\mathrm{H_2O}} \underset{\text{respiration (in light and dark)}}{\overset{\text{photosynthesis (in light)}}{\rightleftharpoons}} \underset{\text{carbohydrate}}{[\mathrm{CH_2O}]_n} + \underset{\text{oxygen}}{n\mathrm{O_2}}$$

The right-to-left reaction in the equation above emphasises that, like all other aerobic organisms, plants carry out respiration, which is essentially the reverse of photosynthesis.

In essence, photosynthesis uses light energy generated by nuclear fusion (fusion of atomic nuclei) in the Sun, to produce 'excited' electrons which can be used for reduction reactions. In higher plants and green algae, photosynthesis takes place in specialised photosynthetic organelles, the chloroplasts, which, like the much smaller mitochondria, lie in the cytosol (Book 1, Section 3.4.11).

Atoms have several discrete energy levels for accommodating electrons, and their lowest 'ground state' energy level is where an electron is most likely to be found. However, an electron can be boosted to a higher energy level by absorbing a photon of light energy. Such an 'excited' electron is highly reactive.

■ Draw and label a rough sketch of a chloroplast, identifying the inner and outer membranes, the stroma and the thylakoids.

□ You should be able to draw a sketch that is a simple version of Figure 3.23b in Book 1. Like the mitochondrion, the chloroplast has an outer and an inner membrane. The space enclosed by the inner membrane is called the stroma and it contains a complex network of membranes known as lamellae. The lamellae form thylakoids – flat sacs, each like a hollow pancake or pitta bread. The thylakoids are arranged in stacks called grana, which are connected by single stromal lamellae.

There are two stages to photosynthesis: the photochemical **light reactions** and the light-independent or **dark reactions** (Figure 3.20). In the light reactions, the energy of the sunlight is used to synthesise ATP and to reduce the coenzyme $NADP^+$ (see Section 3.4 and Table 3.2). These products are then used to drive the dark reactions in which CO_2 is 'fixed' – that is, reduced and converted into carbohydrates.

In the light reactions, light triggers the flow of electrons through an ETC in the chloroplast thylakoid membrane, providing a proton motive force for ATP production. The final acceptor of electrons from the ETC is the coenzyme $NADP^+$. The reduction of $NADP^+$ to NADPH is completed by picking up H^+ ions from the chloroplast stroma. The NADPH and ATP generated by the light reactions are then used in the dark reactions to reduce CO_2 to ultimately form carbohydrates.

But what provides the electrons that are excited by light at the beginning of the ETC? The most abundant electron donor in today's world is H_2O (Figure 3.20). The redox potential (E) of H_2O is +0.82 V, while that of $NADP^+$ is −0.32 V.

■ Which of these molecules has the greatest affinity for electrons?

□ Redox potential is a measure of electron affinity (see Section 3.6.3). Water has a much greater redox potential and therefore a greater affinity for electrons than does $NADP^+$.

The large difference in redox potential (ΔE) between H_2O and $NADP^+$ means that a great deal of energy is needed to drive the transfer of electrons from H_2O to $NADP^+$ and thereby reduce $NADP^+$ to NADPH. (You may find it helpful to look again at Box 3.1 where redox potential and its relation to free energy are explained.)

Light energy is absorbed in discrete packets known as **photons**, and more than one photon of light energy is needed to energise the electrons of H_2O sufficiently for them to be transferred to $NADP^+$. In higher plants, which use H_2O as their electron donor in photosynthesis, this is achieved by having two sets of light-absorbing pigments, allowing electrons to be energised by two photons of light energy. However, in primitive photosynthetic organisms such as the green sulfur bacteria, a different electron donor, H_2S (hydrogen sulfide), which has a much lower redox potential than does H_2O (and hence requires less energy to transfer electrons to $NADP^+$) acts as an electron donor.

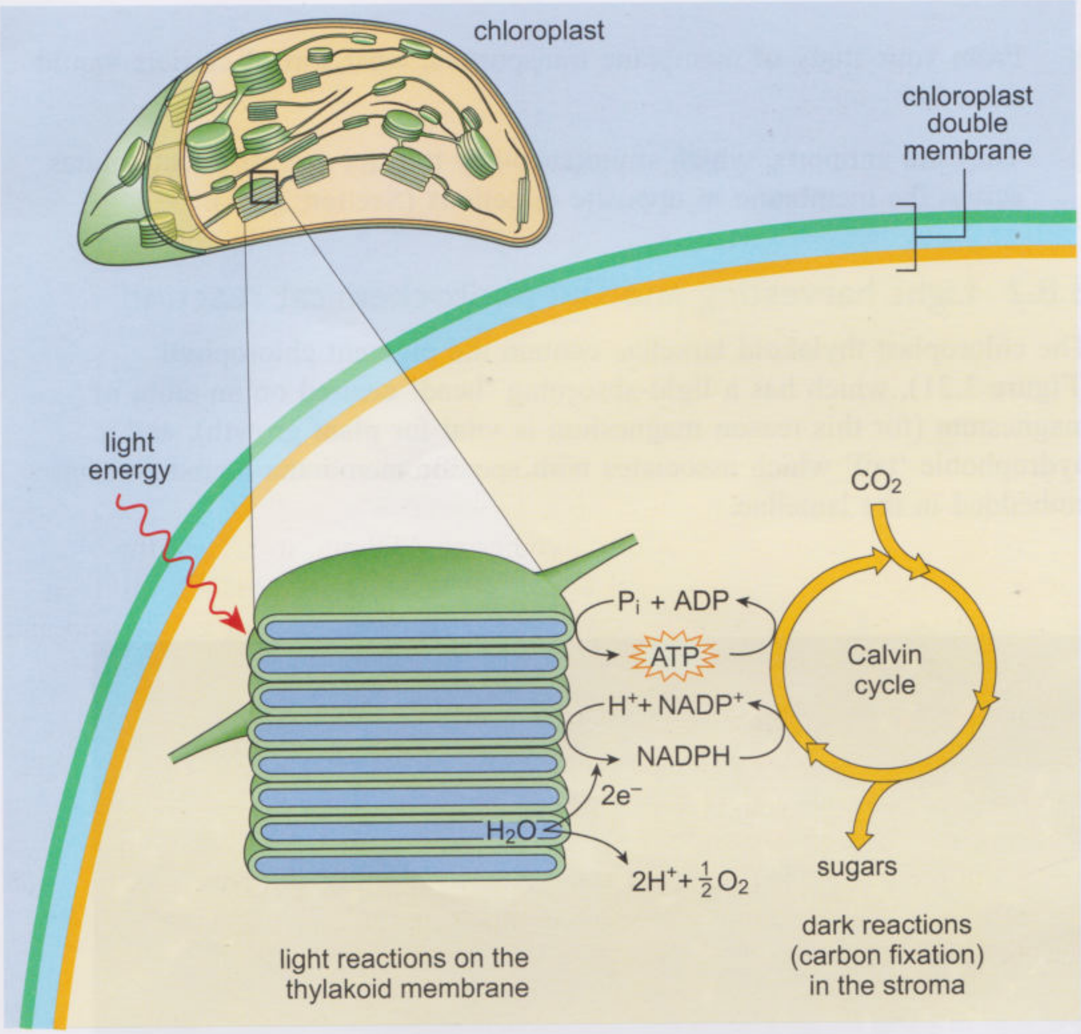


Figure 3.20 The light and dark reactions of photosynthesis.

The next section will look in more detail at photosynthesis in chloroplasts.

3.8.1 Chloroplasts have large areas of internal membrane

When viewed from the side, all that can be seen of the grana of the chloroplast is a pile of membranes, the lamellae (Figure 3.23 in Book 1). The enormous surface area of membrane in the grana is very important in photosynthesis, since the light reactions involve both membrane-bound proteins and pigments that are tethered to the lamellae by their hydrophobic tails. The single (unstacked) stromal lamellae that connect the grana also add to this membrane network. It is in, on and through the thylakoids that light-harvesting, electron flow and proton movement take place in the light reactions of photosynthesis.

While the thylakoid is the site of the light reactions, the chloroplast stroma contains the many enzymes of the dark reactions, which use the ATP and NADPH produced by the light reactions to 'fix' CO_2 by reducing it. The chloroplast envelope comprises a double membrane. It is the selective permeability of these membranes, in particular the inner membrane, which controls the supply of the carbon products to the cytoplasm and to the rest of the plant, and a number of transporters in this membrane export carbon products from the stroma in exchange for inorganic phosphate.

■ From your study of membrane transporters, what sort of carriers would these be?

□ They are antiports, which simultaneously transfer two different solutes across the membrane in opposite directions (Section 2.8.1).

3.8.2 Light harvesting and the photochemical reaction

The chloroplast thylakoid lamellae contain the pigment chlorophyll (Figure 3.21), which has a light-absorbing 'head' centred on an atom of magnesium (for this reason magnesium is vital for plant growth), and a hydrophobic 'tail' which associates with specific membrane-bound proteins embedded in the lamellae.

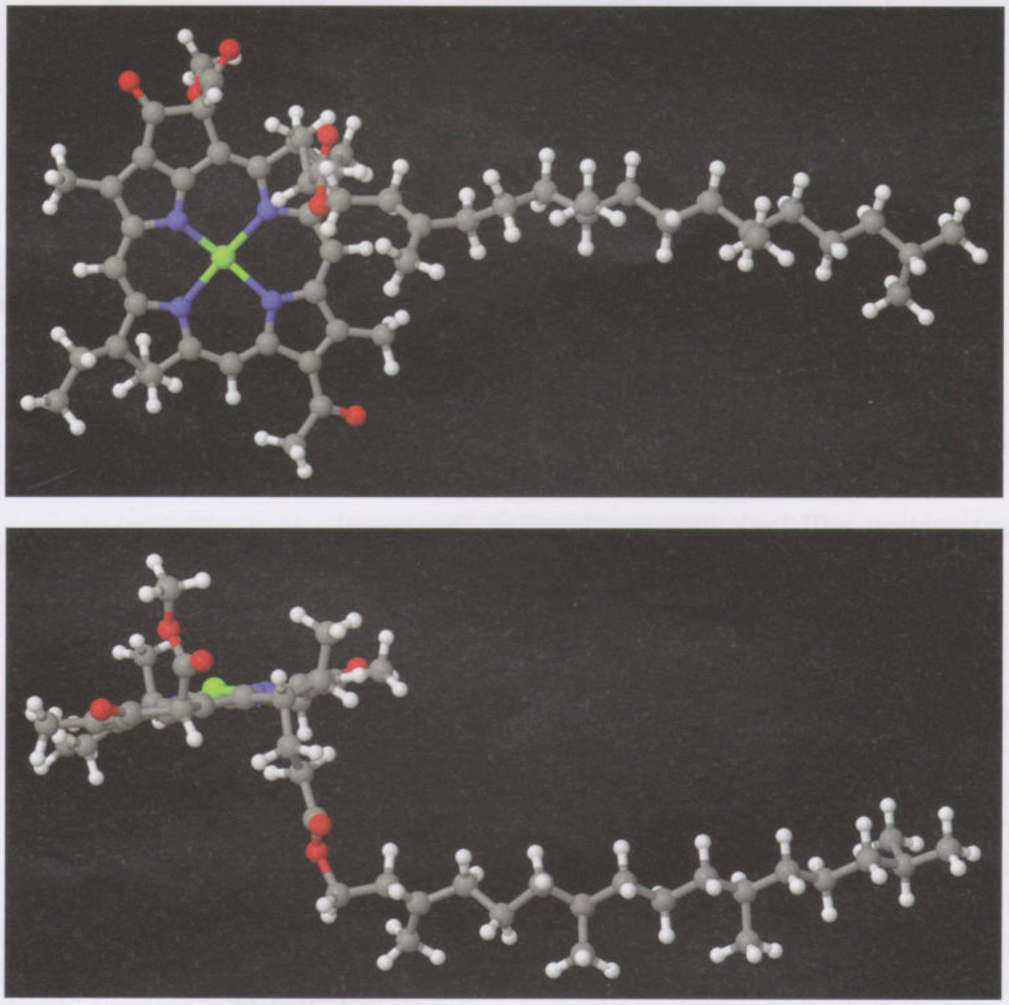


Figure 3.21 Ball-and-stick representation of a chlorophyll molecule displayed in two orientations to show the planar 'head', which is the part of the molecule that absorbs light energy, and the hydrophobic tail, which associates with proteins in the chloroplast lamellar membrane. The atoms are coloured according to convention: magnesium, green; nitrogen, blue; oxygen, red; carbon, grey; hydrogen, white.

Chlorophyll appears green because it absorbs visible light of all wavelengths except those in the green part of the spectrum (which are reflected). Light is absorbed as packets of energy or photons, at the rate of about one photon per chlorophyll molecule per second, causing the chlorophyll to become excited (i.e. boosting an electron in a particular atom in the chlorophyll molecule to a higher energy level).

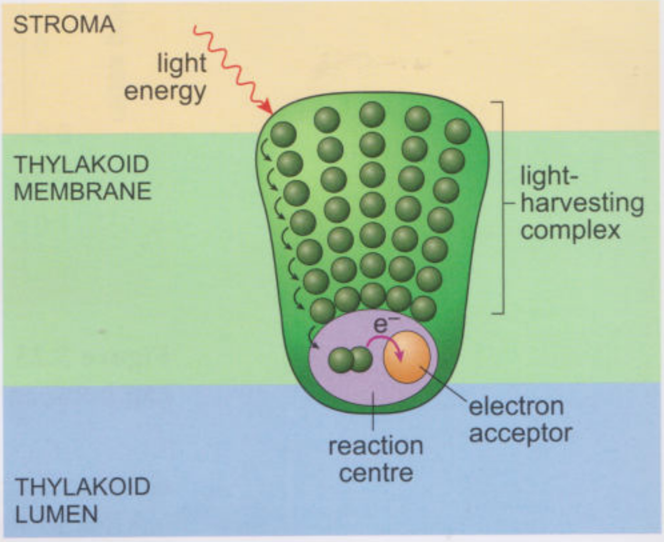


Figure 3.22 Representation of a photosystem showing the movement of energy and electrons from the light-harvesting complex to the reaction centre. The dark green spheres represent chlorophyll molecules. Light energy absorbed by pigments in the LHC is converted into excitation energy, which is then passed from one chlorophyll molecule to the next (small black arrows), and is funnelled towards a specific pair of chlorophyll molecules in the reaction centre. A high-energy electron is ejected (pink arrow) from each of the reaction centre chlorophyll molecules and passes to an electron acceptor. As described in the text, there are two types of photosystem in the thylakoid membrane, termed PSI and PSII, with different electron acceptors.

Light harvesting and energy capture take place in special pigment–protein complexes, known as **photosystems**. Photosystems are composed of a **light-harvesting complex (LHC)** and a **reaction centre (RC)**, as represented in Figure 3.22. The LHC contains several hundred pigment molecules. For plants growing in normal light, up to 60% of the total leaf chlorophyll is found in the light-harvesting complexes. The pigments are arranged in organised arrays so that the excitation energy that results from capture of light energy can be readily passed from one pigment molecule to another, exciting each in turn. The excitation energy is effectively funnelled towards a specific pair of chlorophyll molecules in the reaction centre. These reaction centre chlorophylls, though chemically identical to chlorophylls elsewhere in the photosystem, are excited with less energy input, because of their particular environment (their location and association with other molecules). The excitation energy transferred to the reaction centre chlorophylls from the LHC is sufficient to eject an electron from each. This is the photochemical reaction. The electrons now carry the energy from the absorbed light and are passed to an electron acceptor within the reaction centre. As you will see, what happens to the electrons at this stage depends on the particular photosystem in which they arose.

As was noted earlier, there is not enough energy in one photon to drive the transfer of electrons from water to $NADP^+$ (i.e. to drive the reduction of $NADP^+$ by H_2O). This problem is overcome in green plants by having *two* linked photosystems, each with their own LHC and reaction centre, which operate in series (i.e. electrons move through one, then the other). For historical reasons, the first photosystem is known as PSII (PS-two), and a second photosystem (known paradoxically as PSI or PS-one) gives the electrons a further boost of light energy (Figure 3.23). The two photosystems absorb light at slightly different wavelengths, and are located in different areas of the chloroplast lamellar system. The second energy boost in PSI is enough to shift the redox potential of the protein–pigment carriers to which the electrons are attached to −1.0 V, which is sufficiently negative for electrons to reduce $NADP^+$.

3.8.3 Electron transport, $NADP^+$ reduction and ATP synthesis

Electrons are not passed directly from PSII to PSI. An electron transport chain links the two photosystems and the electrons are transferred via a series of

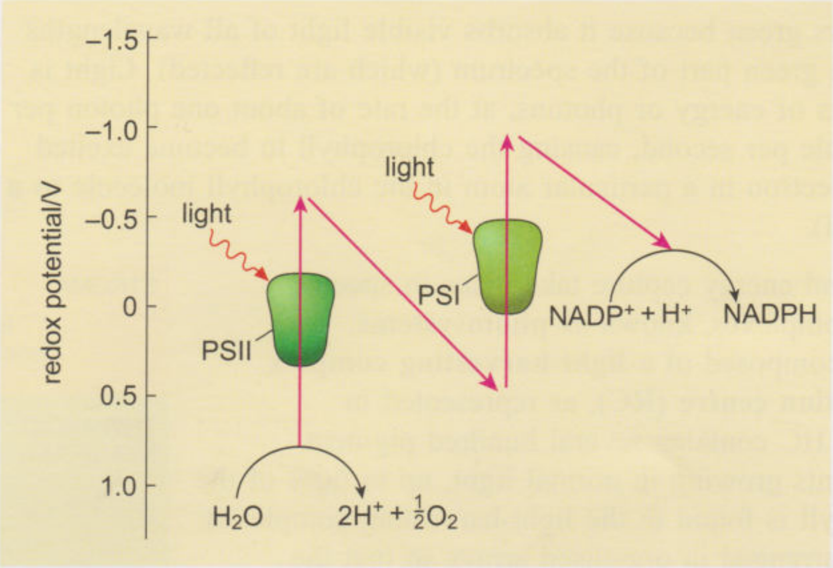


Figure 3.23 Two linked photosystems are needed to bridge the redox potential gap between H_2O and $NADP^+$.

electron carriers in much the same way as electrons released on oxidation of NADH in mitochondria are passed along the mitochondrial ETC. Figure 3.24 shows the components and pathway of photosynthetic electron transport in green plants.

Light energy absorbed by chlorophyll molecules in the LHC of photosystem II (PSII) is converted into excitation energy and is funnelled to the reaction centre as described above. For each photon absorbed, an electron is transferred to an electron acceptor in PSII and is replaced by an electron donated by water. Two electrons are transferred per molecule of water oxidised, as indicated in Figure 3.24.

As in the mitochondrial ETC, small mobile carriers move between large protein complexes. Plastoquinone (PQ in Figure 3.24) picks up the two electrons from PSII (the reduced form of PQ is represented as PQH_2 in Figure 3.24) and delivers them to a cytochrome complex. For every electron that plastoquinone delivers from PSII to the cytochrome complex, it picks up a proton from the stroma and releases it into the thylakoid lumen. From the cytochrome complex, a second mobile carrier, plastocyanin (PC in Figure 3.24), transfers electrons to PSI, where they replace the electrons expelled by the PSI reaction centre.

The electrons from PSI now at last have enough energy to reduce $NADP^+$. They pass via the carrier *ferredoxin* to a membrane-bound protein on the stromal face of the thylakoid membrane, called NADP reductase. NADP reductase catalyses the reduction of $NADP^+$ to NADPH, using the electrons from PSI and drawing protons (H^+) from the chloroplast stroma.

■ Movement of electrons between PSII and PSI is spontaneous; what can you say about the redox potential of successive carriers in the electron transport chain that links the photosystems?

□ Each successive carrier has a more positive redox potential (higher affinity for electrons) than the previous one.

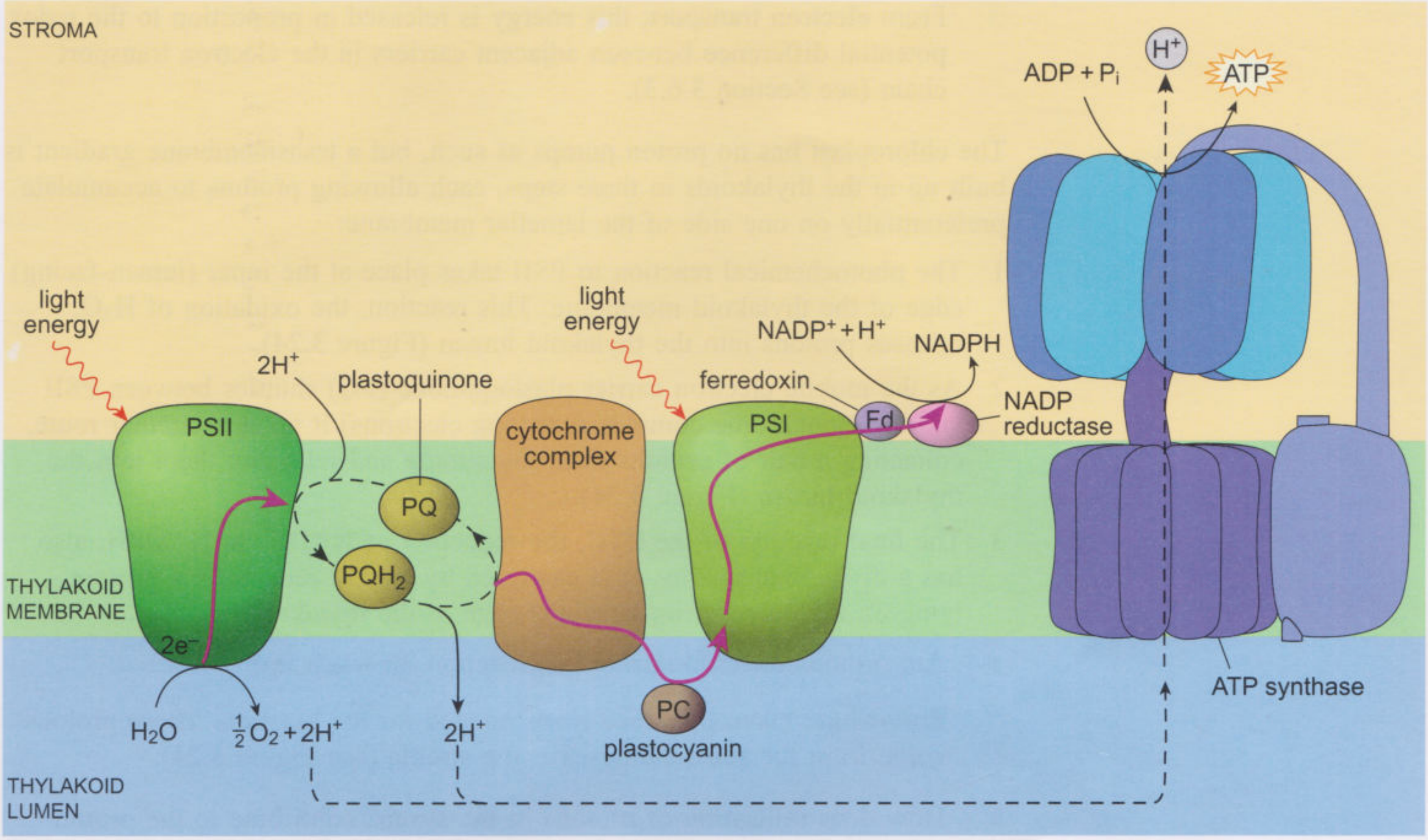


Figure 3.24 The components and pathway of electron transport (indicated by the pink arrows) and photophosphorylation in green plants. The process is described in the text.

Note that there are some striking similarities between the photosynthetic ETC in chloroplasts (Figure 3.24) and the mitochondrial ETC (Figure 3.14). The mobile electron carrier plastoquinone is similar to ubiquinone; the cytochrome complex resembles complex III of the mitochondrial ETC; and the second mobile carrier, plastocyanin, which transfers electrons to PSI, is equivalent to cytochrome *c* in mitochondria.

As in mitochondria, ATP synthesis in chloroplasts is driven by chemiosmotic coupling, using the energy released as protons flow spontaneously down an electrochemical gradient. The proton gradient generated during electron transport (with protons accumulating in the interior or lumen of thylakoids) is discharged via an ATP synthase and used to power ATP synthesis.

■ What component(s) of mitochondria enable them to build a transmembrane proton gradient by moving protons to one side of the membrane?

□ Three of the respiratory complexes (complexes I, III and IV) can pump protons out of the matrix into the intermembrane space (see Figure 3.14).

■ Where do proton pumps get the energy needed to 'pump' protons against an electrochemical gradient in mitochondria?

□ From electron transport; this energy is released in proportion to the redox potential difference between adjacent carriers in the electron transport chain (see Section 3.6.3).

The chloroplast has no proton pumps as such, but a transmembrane gradient is built up in the thylakoids in three steps, each allowing protons to accumulate preferentially on one side of the lamellar membrane:

1 The photochemical reaction in PSII takes place at the inner (lumen-facing) edge of the thylakoid membrane. This reaction, the oxidation of H_2O, releases protons into the thylakoid lumen (Figure 3.24).

2 As the mobile electron carrier plastoquinone (PQ) shuttles between PSII and the cytochrome complex (carrying electrons) it takes a circular route, collecting a pair of protons from the stroma and releasing them into the thylakoid lumen (Figure 3.24).

3 The final reaction of the ETC, the reduction of $NADP^+$ to NADPH, also has a directional quality. It is catalysed by NADP reductase, a protein lying on the outer (stroma-facing) edge of the thylakoid lamella.

■ Are protons released during this reaction, or taken up?

□ Protons are taken up, since H^+ is needed for the reaction. These protons come from the surroundings, i.e. the stroma (see Figure 3.24).

■ How does utilisation of protons in the stroma contribute to the proton gradient across the thylakoid membrane?

□ It reduces the concentration of protons on the stromal side of the membrane relative to the thylakoid lumen.

So, with passage of light-energised electrons along the ETC in the thylakoid lamella, there are three steps contributing to the establishment of a proton gradient across the thylakoid membrane (protons being concentrated in the thylakoid lumen). In this way, the electron transport energy (derived from light captured by the photosystems) is stored as a transmembrane proton gradient.

The transmembrane proton gradient in chloroplasts drives ATP synthesis (known here as photophosphorylation) via an ATP synthase that is structurally very similar to that in mitochondria. Thus protons flow into the stroma from the thylakoid lumen (i.e. down their electrochemical gradient), through the ATP synthase and the energy stored in the proton gradient is used to drive the synthesis of ATP, as shown on the right in Figure 3.24. The parallels between chemiosmotic coupling of ETC activity and ATP synthesis in mitochondria and chloroplasts are highlighted in Figure 3.25.

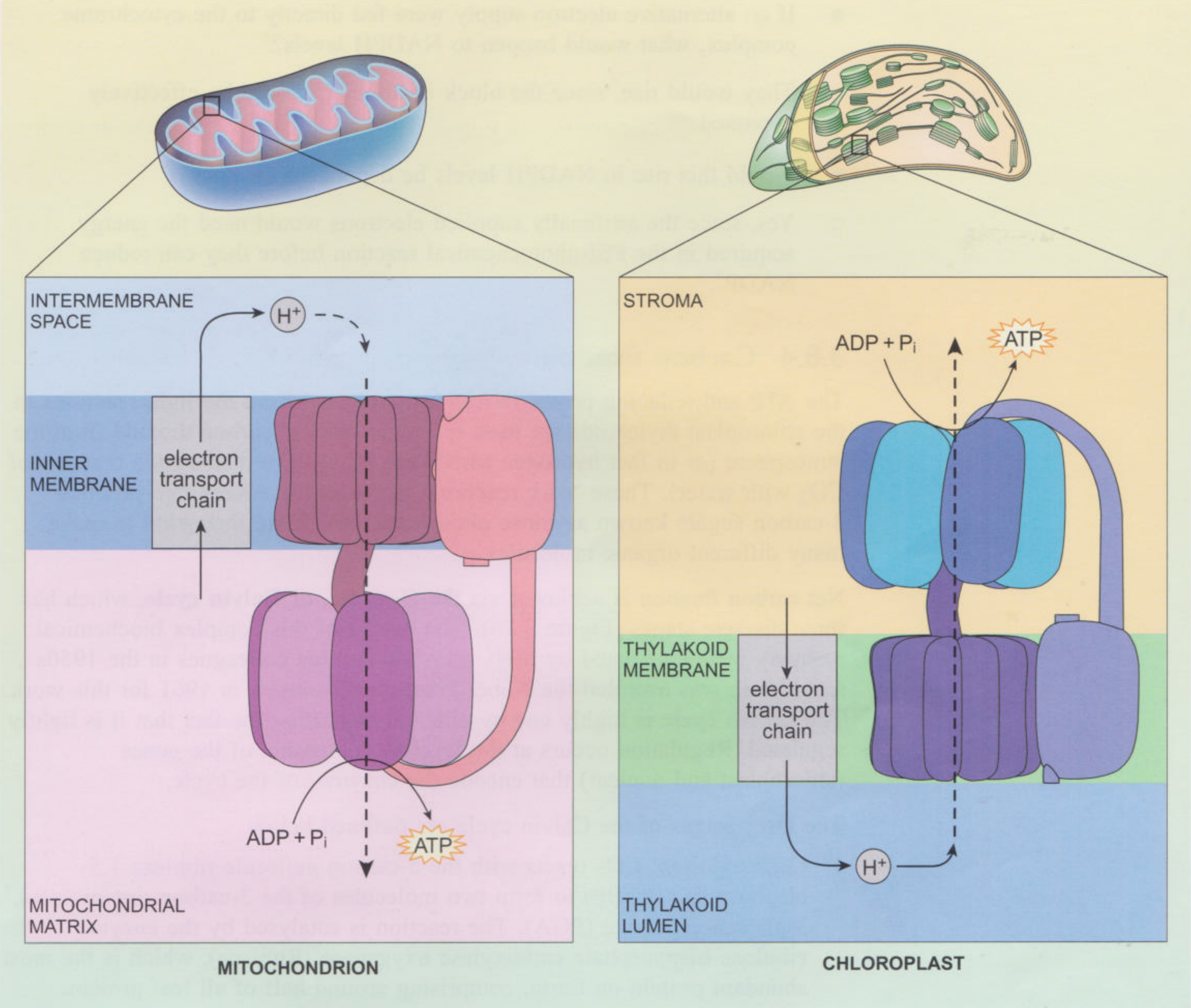


Figure 3.25 Comparison of chemiosmotic coupling in mitochondria and chloroplasts. In both these organelles, electron transport activity generates a proton gradient across a membrane. The energy released when the protons pass down their electrochemical gradient, via ATP synthase, drives ATP synthesis.

Many synthetic herbicides (weedkillers) are directed at the ETC. For example, DCMU (dichlorophenyl dimethylurea) prevents plastoquinone from carrying electrons between PSII and the cytochrome complex, while paraquat accepts electrons from ferredoxin, thus preventing them being used to reduce $NADP^+$. Worse still, the reduced paraquat then reacts with oxygen to form superoxide, a highly reactive lipid-damaging free radical.

Free radicals are atoms, molecules or ions that have unpaired electrons. In most cases, free radicals are highly reactive, and as such can be very damaging when they are generated in biological systems.

- ■ What would you expect to happen to supplies of NADPH in green leaves treated with DCMU?
- □ The NADPH supply would become exhausted, since no electrons could pass down the ETC beyond plastoquinone.

■ If an alternative electron supply were fed directly to the cytochrome complex, what would happen to NADPH levels?

☐ They would rise, since the block in the ETC would be effectively bypassed.

■ Would this rise in NADPH levels be dependent on light?

☐ Yes, since the artificially supplied electrons would need the energy acquired in the PSI photochemical reaction before they can reduce $NADP^+$.

3.8.4 Carbon fixation

The ATP and reducing power (NADPH) generated from the light reactions in the chloroplast thylakoids are used to reduce or 'fix' carbon dioxide from the atmosphere (or in fact hydrogen carbonate, HCO_3^-, formed by the reaction of CO_2 with water). These 'dark reactions' occur in the stroma and produce 3-carbon sugars known as triose phosphates, which are then used to make many different organic molecules.

Net carbon fixation is achieved via the C_3 cycle, or **Calvin cycle**, which has three discrete stages (Figure 3.26). The details of this complex biochemical pathway were elucidated by Melvin Calvin and his colleagues in the 1950s and Calvin was awarded the Nobel Prize for Chemistry in 1961 for this work. The Calvin cycle is highly energy efficient, reflecting the fact that it is tightly regulated. Regulation occurs at the level of expression of the genes (chloroplast and nuclear) that encode the enzymes of the cycle.

The three stages of the Calvin cycle are outlined below.

1. *Carboxylation*: CO_2 reacts with the 5-carbon molecule ribulose 1,5-bisphosphate (RuBP) to form two molecules of the 3-carbon organic acid, 3-phosphoglycerate (PGA). The reaction is catalysed by the enzyme **ribu**lose **bis**phosphate **c**arboxylase **o**xygenase (Rubisco), which is the most abundant protein on Earth, comprising around half of all leaf protein.
2. *Reduction*: The next step, reduction of PGA to a 3-carbon sugar phosphate (i.e. triose phosphate), requires a large input of energy. This is derived from NADPH and ATP.
3. *Regeneration*: For more molecules of CO_2 to be fixed, there is a need to regenerate the initial carbon acceptor molecule, RuBP. This is achieved in a complex series of reactions involving 3-, 4-, 5-, 6- and 7-carbon sugar phosphates, requiring more ATP. For the purposes of this module, you need not concern yourself with the details of these steps.

In steady-state photosynthesis, most (five-sixths) of the triose phosphate molecules produced by carbon fixation are used to regenerate RuBP. Carbon fixed in excess of that needed to regenerate RuBP is used within the chloroplast for the synthesis of starch (the principal storage form of carbohydrate), lipids, amino acids, etc., or is exported from the chloroplast as triose phosphate. Product synthesis is very versatile and depends on the environmental conditions, the stage of growth of the photosynthetic tissue and the metabolic needs of the plant. Exported triose phosphate is mainly

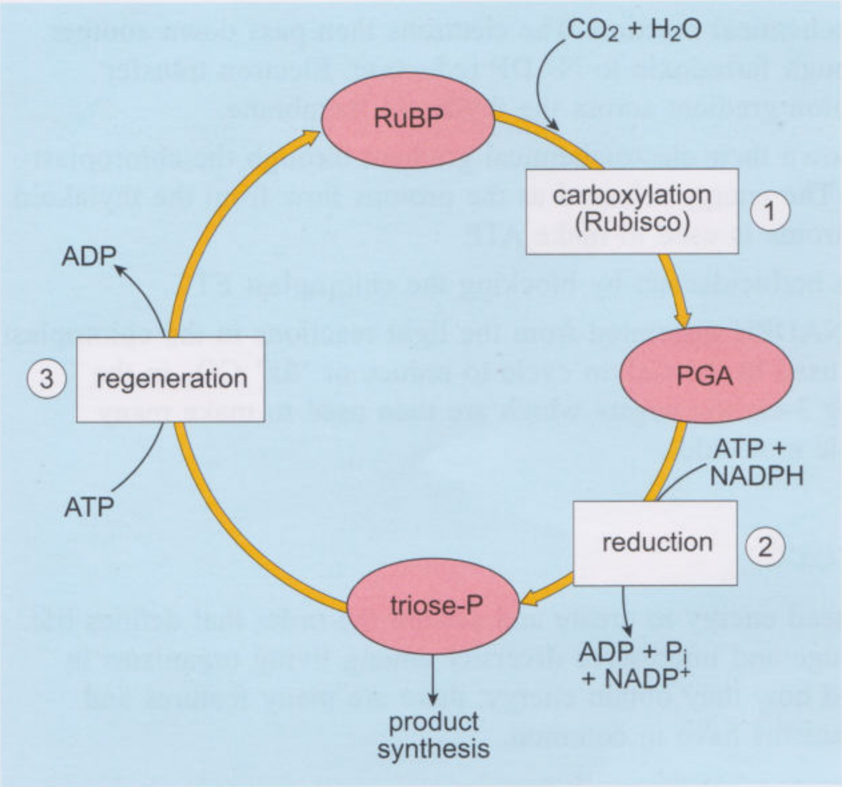


Figure 3.26 A highly simplified summary of the C_3 or Calvin cycle, showing the three stages of photosynthetic carbon reduction (labelled 1–3). RuBP is ribulose 1,5-bisphosphate and PGA is 3-phosphoglycerate.

consumed in sucrose synthesis in the cytoplasm. Sucrose is the mobile product of photosynthesis that powers growth and metabolism throughout the plant body.

Remarkably, virtually all the organic compounds that modern organisms require are produced by photosynthesis in plants, algae and photoautotrophic bacteria.

Summary of Section 3.8

- Photophosphorylation is a form of chemiosmotic coupling in which electrons are energised by light rather than by metabolism. The purple-membrane archaeon *Halobacterium halobium* has a light-powered proton pump, able to make ATP by chemiosmotic coupling.
- In higher plants and green algae, photosynthesis takes place in the chloroplasts. There are two stages to photosynthesis: the light reactions and the dark reactions. In the light reactions, the energy of sunlight is used to make both ATP and reduced coenzyme (NADPH). These products are then used to drive the dark reactions in which CO_2 is reduced and converted into carbohydrates and other molecules.
- In chloroplasts, light energy is absorbed by two linked photosystems; in this way, electrons from the electron donor, water, are given two energy 'boosts', enabling them to reduce $NADP^+$.
- In chloroplasts, energised electrons from the first photochemical reaction, in PSII, pass along an ETC to PSI where they replace electrons ejected in

a second photochemical reaction. The electrons then pass down another short ETC through ferredoxin to NADP reductase. Electron transfer generates a proton gradient across the thylakoid membrane.

- Protons flow down their electrochemical gradient through the chloroplast ATP synthase. The energy released as the protons flow from the thylakoid lumen to the stroma is used to make ATP.
- Many common herbicides act by blocking the chloroplast ETC.
- The ATP and NADPH generated from the light reactions in the chloroplast thylakoids are used in the Calvin cycle to reduce or ‘fix’ CO_2 in the stroma, forming 3-carbon sugars which are then used to make many different organic molecules.

3.9 Final word

All living things need energy to create and sustain the order that defines life. While there is a huge and impressive diversity among living organisms in terms of where and how they obtain energy, there are many features and strategies that organisms have in common.

Activity 3.2 Making ATP

(LOs 3.6, 3.7 and 3.8) Allow 2 hours

This activity is designed to help you to understand what happens during oxidative phosphorylation in mitochondria and photophosphorylation in chloroplasts. It should further help you to appreciate the principle of chemiosmotic coupling, which is common to both these processes.

3.10 Learning outcomes

3.1 Outline how organisms can be categorised according to their energy source and carbon source.

3.2 Understand and explain basic chemical principles that relate to metabolism.

3.3 Understand and describe what is meant by a metabolic pathway and discuss different mechanisms by which metabolism is controlled.

3.4 Outline how ATP and other coenzymes function as energy carriers.

3.5 Describe, with the help of simple diagrams, glycolysis, the link reaction and the TCA cycle, identifying where, and how much, ATP and reduced coenzymes are generated.

3.6 Outline the principles of the chemiosmotic coupling model and describe how proton pumping links electron transport and ATP synthesis in mitochondria.

3.7 Describe, with the help of simple diagrams, the light and dark reactions of photosynthesis.

3.8 Compare and contrast electron transport, generation of an electrochemical proton gradient and chemiosmotic coupling in mitochondria and chloroplasts.

Chapter 4 Cell communication

4.1 Introduction

Every living cell needs to sense and respond to its surroundings in order to survive and function. Both single-celled organisms and the individual cells of multicellular organisms live in complex environments and are constantly bombarded with a host of signals, both chemical and physical. Such signals have the potential to induce changes in the cell's behaviour: for example, in a bacterium that moves in the direction of nutrients (Chapter 5); or in an animal or plant cell undergoing differentiation in a developing embryo (Book 3, Chapter 1). Indeed, in some cases, the survival of a cell depends on the presence of a particular signalling molecule.

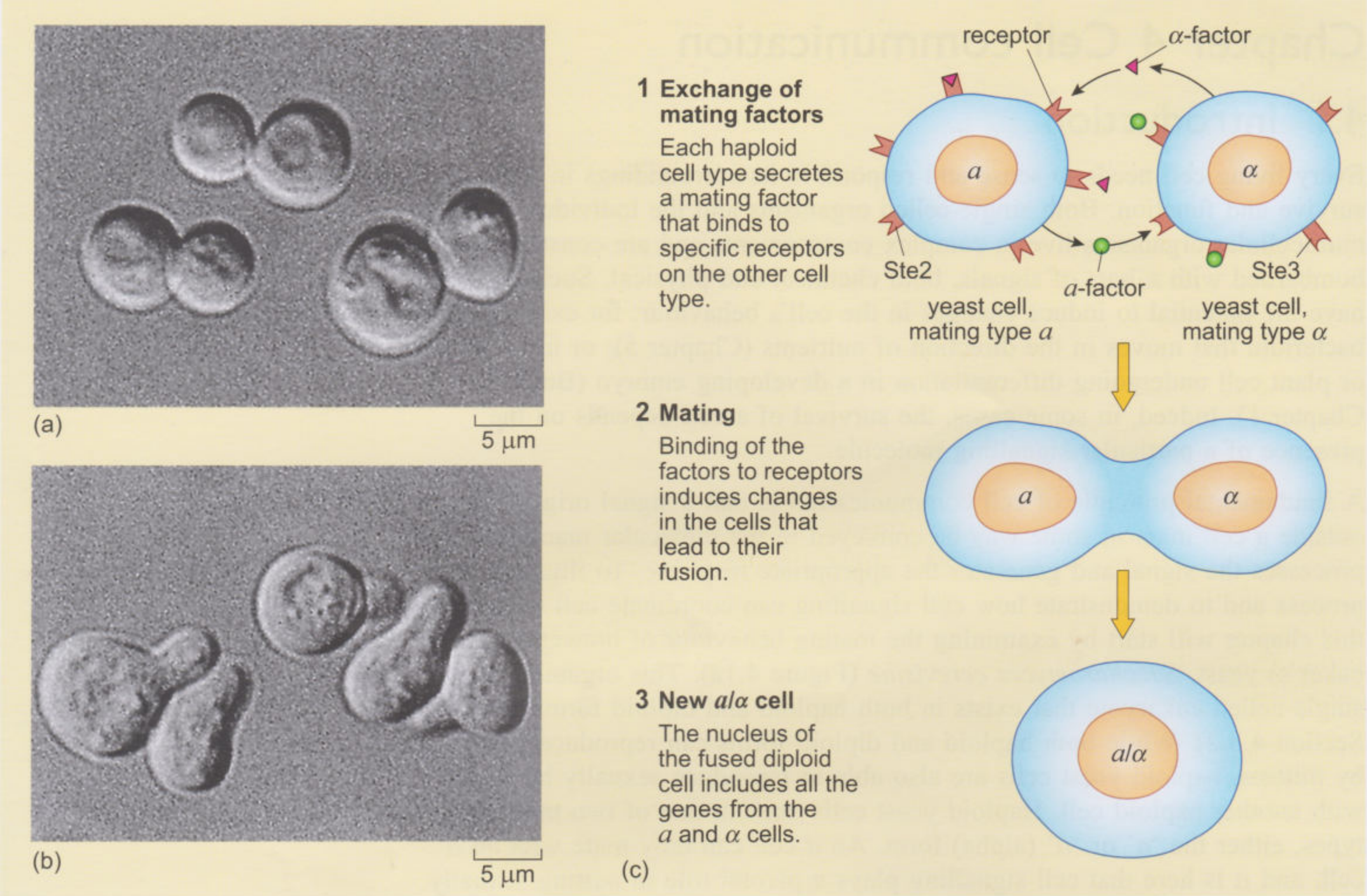
A fundamental principle of cell communication is that a signal originating outside a cell must in some way be conveyed to the molecular machinery that processes the signal and generates the appropriate response. To illustrate this process and to demonstrate how cell signalling can coordinate cell behaviour, this chapter will start by examining the mating behaviour of brewer's (or baker's) yeast, *Saccharomyces cerevisiae* (Figure 4.1a). This organism is a single-celled eukaryote that exists in both haploid and diploid forms (Book 1, Section 4.3.2). While both haploid and diploid forms can reproduce asexually by mitosis, haploid yeast cells are also able to reproduce sexually by fusing with another haploid cell. Haploid yeast cells exist in one of two mating types, either the '*a*' or 'α' (alpha) form. An *a* cell can only mate with an α cell, and it is here that cell signalling plays a pivotal role in pairing sexually compatible yeast cells. Cells of the *a* mating type secrete a mating pheromone, a peptide molecule called *a*-factor, into their local surroundings; while α cells secrete a different mating pheromone called α-factor. These mating factors bind to cell surface receptors on an appropriate target cell (Figure 4.1c). Crucially, only *a* cells express the type of cell surface receptor (called the Ste2 receptor) that can specifically recognise and bind the α-factor. Conversely, only α cells express the cell surface receptor that recognises *a*-factor (the Ste3 receptor). It is important to note that these receptors do not recognise or bind the mating factor released by the cell on which they are located; instead, they can only bind the factor released by a cell of the opposite mating type. This specificity of the relationship between a signalling molecule and its target receptor is a fundamental aspect of cell communication.

A pheromone is a chemical factor released by an individual that can potentially trigger a change in the behaviour of other individuals of the same species.

In *S. cerevisiae*, the binding of the appropriate pheromone to the cell surface receptor activates the mating response. Compatible cells grow projections towards each other (as illustrated in Figure 4.1b) and fuse, thus creating a diploid cell (*a*/α) which, depending on environmental circumstances, will either divide by mitosis to produce a colony of diploid *a*/α cells or will undergo meiosis and sporulation to form haploid α and *a* spores.

The mating of *S. cerevisiae* demonstrates the four key features of cell communication:

1 *Signal.* A signal must first be present to initiate cell communication. In the example of mating yeast, the signal is a peptide known as the mating



1 Exchange of mating factors

Each haploid cell type secretes a mating factor that binds to specific receptors on the other cell type.

2 Mating

Binding of the factors to receptors induces changes in the cells that lead to their fusion.

3 New *a*/*α* cell

The nucleus of the fused diploid cell includes all the genes from the *a* and *α* cells.

Figure 4.1 An example of a cellular response to the activation of a signalling pathway by an extracellular molecule. (a) Resting yeast cells. (b) Yeast cells respond to mating factor by extending protrusions towards the cell that releases the appropriate mating factor. (c) Schematic illustrating the mating of two compatible haploid yeast cells to produce a diploid cell.

pheromone. However, as you will learn later in this chapter, signalling molecules are diverse in nature.

2 *Reception*. In order to elicit a response, the signal has to bind to a receptor. The yeast mating factor receptor is located on the exterior cell surface; however, some signalling molecules bind to receptors located inside the cell.

3 *Transduction*. When the signal binds to its receptor, it causes the receptor to change in some way, triggering **signal transduction**, the process by which the signal is converted into a form that can bring about a specific cellular response.

4 *Response*. The transduced signal ultimately elicits a response (i.e. some change in activity) in the target cell. In the case of mating yeast, the response is the growth of a projection that allows the mating cells to fuse.

Yeast, like most single-celled organisms, secretes signalling molecules into the local environment. Complex multicellular organisms require a more sophisticated range of intercellular signalling mechanisms. In an animal, for example, the activities of the different tissues and organs must be coordinated, the chemical components of the internal environment maintained within a narrow physiological range, and crises, such as wounds or infections, must be dealt with appropriately. Some responses, such as movement, must be fast; others, such as growth, may be slow. A variety of specialised cells, tissues and systems, and a wealth of signalling molecules and receptors have evolved that enable these requirements to be met.

An important point to recognise, which is illustrated by the yeast mating example, is that cells only respond to a signal if they express the corresponding receptor. While there may be thousands of different types of receptor expressed in the different tissues of a multicellular organism such as a human, any particular cell will only express a subset of these and hence be sensitive to the corresponding signals (though of course the profile of receptors expressed by a cell is subject to change in the lifetime of the cell). Despite this huge variety in receptors, a relatively small number of different intracellular signal transduction mechanisms are responsible for communicating the signal inside the cell and ultimately eliciting the appropriate cellular response. In fact, the complexity of intracellular signalling pathways and the diversity and subtlety of the cell responses that these pathways trigger, arise from the many potential interactions between different pathways.

Signal transduction is one of the areas of cell biology that is currently the focus of intense research. The reason for this interest is, of course, that signal transduction underpins all aspects of cell function and cell physiology. It directs all cell behaviour; not only normal responses to environmental change and the coordination of a cell's activities with those of other cells, tissues and organs, but also the abnormal behaviour that occurs in cancer and many other diseases.

Summary of Section 4.1

- Cells of both single-celled and multicellular organisms communicate by secreting signalling molecules.
- Signalling molecules bind to specific receptors located on (or sometimes within) the target cells.
- When a signalling molecule binds to a receptor, it initiates transduction of the signal into a form that is capable of bringing about a cellular response.
- While there are many different receptor types, the variety in intracellular transduction mechanisms is relatively limited.

4.2 How do cells signal to one another?

The signalling molecules produced by a signalling cell are, in almost all instances, detected by binding to protein receptors on the target cell. The signalling molecules are hence ligands of their receptors (see Chapter 1). Receptors are specific for a particular ligand or a few structurally similar ligands and are located either in the cell membrane or, in a few cases, inside the cell.

■ In Section 4.1, you learnt about how yeast use pheromones to signal to a compatible mate. Identify the ligands and receptors involved in the mating of yeast.

□ The ligands used by yeast are the mating pheromones α-factor and *a*-factor which specifically bind to the cell surface receptors Ste2 and Ste3, respectively.

Some signalling molecules, such as steroid hormones (see Section 4.3.2), are lipid-soluble and can diffuse freely across the cell membrane to act on intracellular receptors. Most signalling molecules, however, are polar and therefore not lipid-soluble; such signalling molecules are unable to cross the lipid bilayer of the cell membrane. Their receptors are located in the cell membrane and when the signalling molecule binds to them (at the extracellular surface) the receptor effectively transforms the signal so that it is communicated to the interior of the cell.

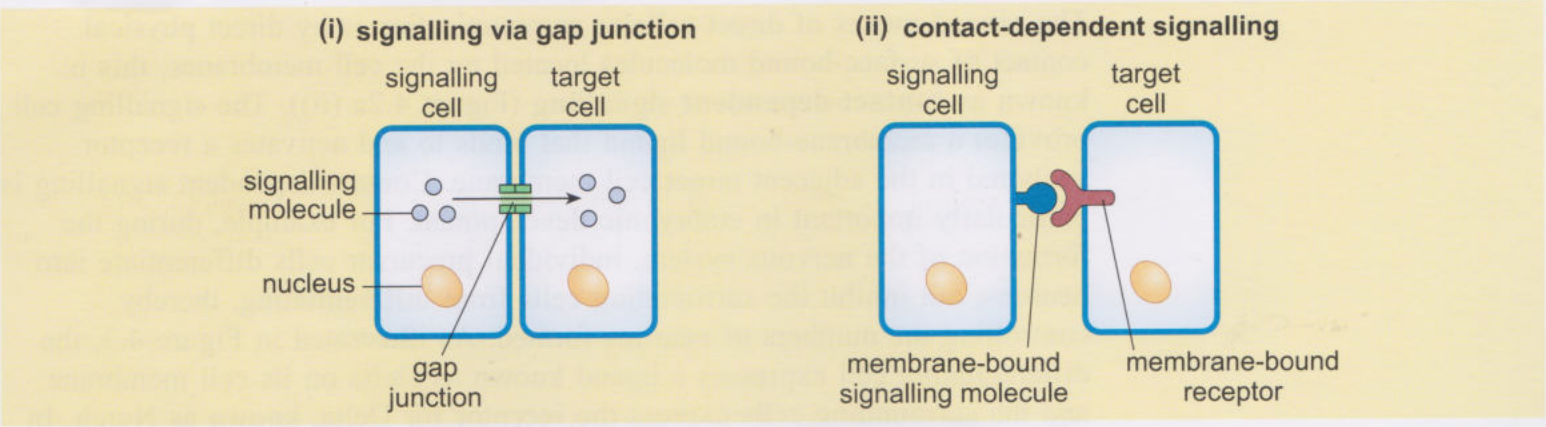
■ What is the term used to describe the transformation of the signal to the interior of the cell where the cellular response is initiated?

□ The term used to describe the transformation of the signal is 'transduction'.

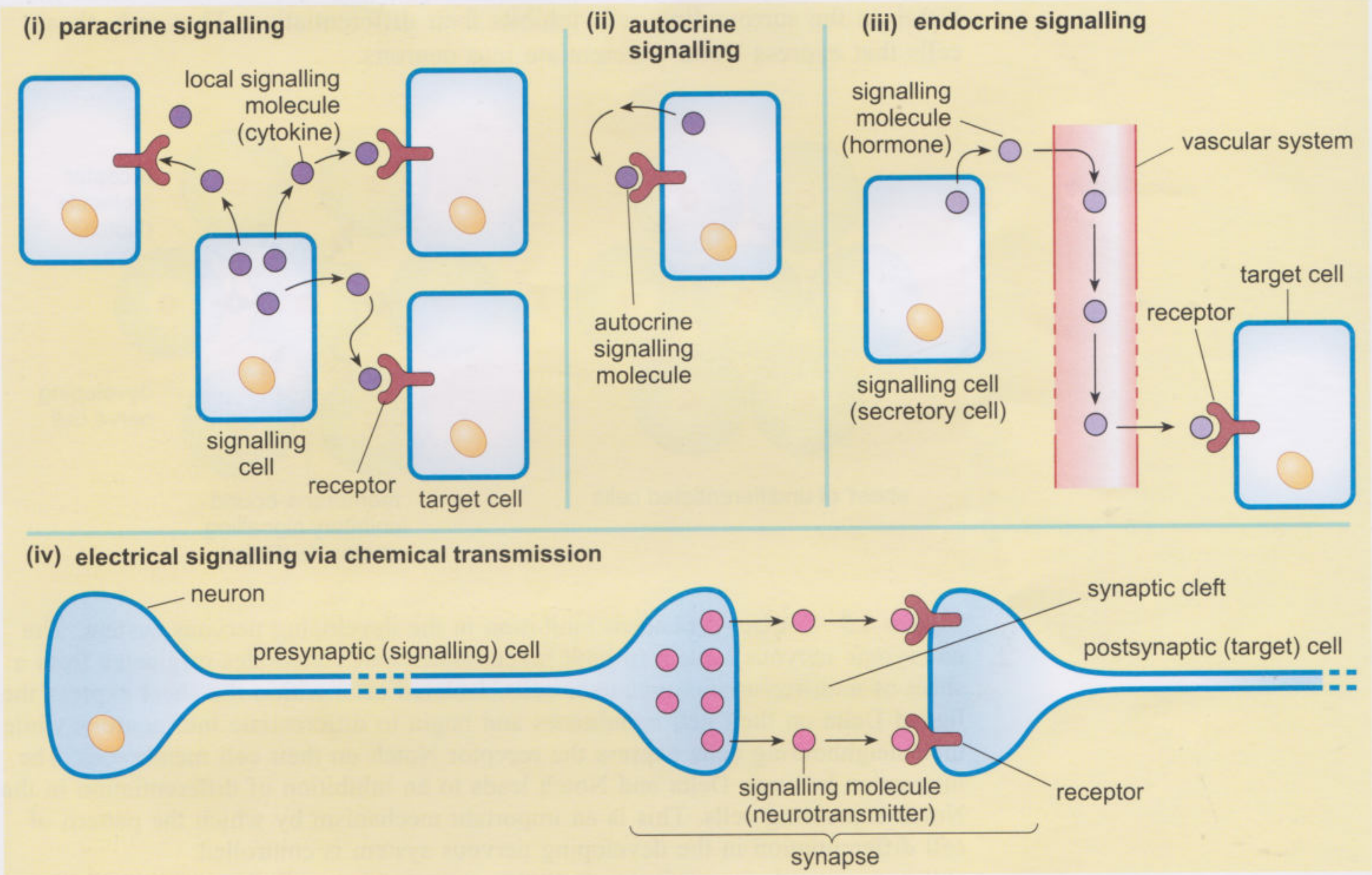
A variety of intercellular signalling strategies exist in animals, as illustrated in Figure 4.2, and these are generally readily distinguished according to the distance between the signalling cell and the target cell. Cells close to each other are able to use physical contact, whereas others secrete signalling molecules that act locally. In more complex organisms, some specialised cells use the blood circulation to transport their signalling molecules to the target and others use electrical impulses to convey signals at rapid speeds and to allow complex coordinated movement. Sections 4.2.1 to 4.2.5 describe these signalling strategies in more detail.

4.2.1 Signalling that depends on cell–cell contacts

The simplest way that cells can communicate with one another is through physical contact. Communication can then take place in one of two main ways (Figure 4.2a). The first of these is via specialised channels that establish a physical conduit between the adjoining cells. Gap junctions (introduced in Book 1, Section 3.5.2) can allow ions and very small molecules such as amino acids, small peptides and simple sugars (monosaccharides) to pass from one cell to another. They also allow passage of electric current, a form of signalling involved in the coordinated contraction of some muscles, such as those responsible for the contraction of the heart.



(a) CELL–CELL CONTACT-DEPENDENT SIGNALLING


(i) paracrine signalling
local signalling molecule (cytokine)
signalling cell
receptor
target cell
(ii) autocrine signalling
autocrine signalling molecule
(iii) endocrine signalling
signalling molecule (hormone)
vascular system
target cell
receptor
signalling cell (secretory cell)
(iv) electrical signalling via chemical transmission
neuron
presynaptic (signalling) cell
synaptic cleft
postsynaptic (target) cell
receptor
signalling molecule (neurotransmitter)
synapse

(b) CELL–CELL SIGNALLING VIA SECRETED MOLECULES

Figure 4.2 The major types of signalling mechanism found in multicellular organisms. (a) Signalling that depends on contact between cells: (i) via gap junctions; (ii) via cell surface molecules, in which both the ligand and the receptor are located on the cell membrane of the signalling cell and the target cell, respectively. (b) Signalling that depends on secreted molecules (which are mainly water-soluble). (i) Paracrine signalling, in which signalling molecules (for example, cytokines) are released and act locally on nearby cells. (ii) Autocrine signalling, in which signalling molecules are released and then act on the cell that produced them. (iii) Endocrine signalling, in which signalling molecules (hormones) are released from specialised cells and carried in the vascular system (blood circulation) to act on target cells at some distance from the site of release; depending on the nature of the ligand, the receptor can be on the membrane (as shown here) or be intracellular. (iv) Electrical signalling, in which the signalling cell transmits information in the form of changes in membrane potential along the length of the cell; the electrical signal is transferred from the signalling cell (here a neuron) to the target cell, either in chemical form as a neurotransmitter (shown here) or via gap junctions (not shown).

The second means of direct cellular communication is by direct physical contact of surface-bound molecules located on the cell membranes; this is known as **contact-dependent signalling** (Figure 4.2a (ii)). The signalling cell provides a membrane-bound ligand that binds to and activates a receptor anchored in the adjacent target cell membrane. Contact-dependent signalling is particularly important in embryonic development. For example, during the formation of the nervous system, individual precursor cells differentiate into neurons, but inhibit the surrounding cells from differentiating, thereby controlling the numbers of neurons formed. As illustrated in Figure 4.3, the differentiating cell expresses a ligand known as Delta on its cell membrane and the surrounding cells express the receptor for Delta, known as Notch. In an example of what is known as contact-dependent *inhibition*, activation of Notch in the surrounding cells inhibits their differentiation. Thus only those cells that express Delta differentiate into neurons.

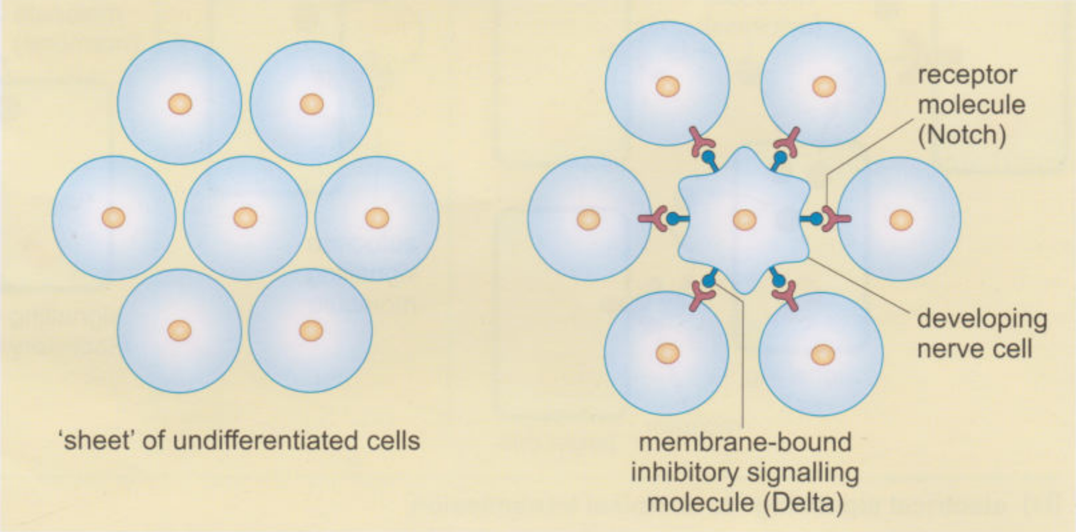

'sheet' of undifferentiated cells
receptor molecule (Notch)
developing nerve cell
membrane-bound inhibitory signalling molecule (Delta)

Figure 4.3 Contact-dependent inhibition in the developing nervous system. The embryonic nervous system in both invertebrates and vertebrates originates from a sheet of undifferentiated precursor cells. Isolated cells within the sheet express the ligand Delta on their cell membranes and begin to differentiate into neurons while their neighbouring cells express the receptor Notch on their cell membranes. The interaction between Delta and Notch leads to an inhibition of differentiation in the Notch-expressing cells. This is an important mechanism by which the pattern of cell differentiation in the developing nervous system is controlled.

4.2.2 Paracrine and autocrine signalling

In many cases, cells release signalling molecules that act on nearby cells or cells located at some distance from the source of the signal. If the signalling molecule is released but then very rapidly destroyed by extracellular enzymes, or rapidly taken up by other cells, then the distance over which the signal travels is relatively short. This local type of signalling is known as **paracrine** signalling (Figure 4.2b (i)). Many signalling molecules act locally: for example, those that initiate inflammation at sites of infection, or stimulate cell proliferation (cell division) at wounds. Note, however, that local signalling is also essential in normal, uninjured tissues.

■ Why would paracrine signalling at the site of an infection or wound be advantageous?

□ Paracrine signalling has the advantage of confining the cellular response (inflammation) to the small area where it is needed.

A major group of locally acting molecules is the **cytokines**, small protein molecules commonly used as intercellular signalling molecules by the cells of the immune system. Activation of different types of cytokine receptors results in a wide range of cellular responses such as secretion of other signalling mediators, the promotion of growth, proliferation and movement. There are many different cytokines, many of which act only on specific types of cell. There are also numerous receptors for individual cytokines, and some cytokines can activate as many as four or five types of receptor. Only cells that express the receptors for a particular cytokine respond to that cytokine. However, since a cytokine may bind to more than one type of receptor, each activating different intracellular signalling pathways, a single cytokine could potentially initiate a host of different responses in different cells.

Growth factors are another major group of small locally acting signalling proteins. Examples include epidermal growth factor (EGF), which stimulates growth and division of epithelial cells, and nerve growth factor (NGF), which stimulates the survival, growth and differentiation of some neurons. Again, there are many different types of factor within this group.

Some cells release signalling molecules that act on receptors on their own surface, a mechanism known as **autocrine** signalling (Figure 4.2b (ii)). Autocrine signalling enables cells to amplify a positive signal in a controlled manner. An example is prostaglandin signalling during parturition (labour) in humans and other mammals. Prostaglandins, signalling molecules derived from a group of unsaturated long-chain fatty acids known as *eicosanoids*, which are found in the cell membrane, are released by the smooth muscle cells that are involved in uterine contraction, and act on the same cell to further stimulate contraction. In other cases, autocrine signalling can inhibit the release of the signalling molecule itself; for example, dopamine, a signalling molecule secreted by specialised neurons in the brain, inhibits its own release by acting on receptors located near the site of release. In neurons exhibiting such autocrine signalling, the receptors involved are called *autoreceptors*.

4.2.3 Endocrine signalling

All but the smallest multicellular organisms need to coordinate the activity of cells that are some distance away from each other. One way for cells to communicate at a distance is by secretion and widespread transport of signalling molecules within the organism by means of a vascular system (the blood circulation in vertebrates). In animals, this type of signalling is known as **endocrine** signalling (Figure 4.2b (iii)). The signalling molecules are known as **hormones** and are secreted by endocrine cells, often grouped together into glands. If the signalling molecule is not broken down in the circulation, it has the advantage of being able to deliver the signal to all tissues. Again, the specificity of the signal depends on the presence of the appropriate receptor on the target cell.

The hormone **adrenalin** (also known as **epinephrine**) is released into the blood supply from the adrenal glands, small glands located above the kidneys in mammals. This hormone is released during periods of acute stress and is responsible for the 'fight or flight' response, so called because it readies the body for action in a hazardous situation. Adrenalin produces a wide variety of changes in the body, determined by the type of receptors expressed in particular tissues. In the heart, it acts on β-adrenergic receptors on cardiac muscle cells to increase heart rate and hence increase the supply of blood to the limbs. In the gut, however, it acts on α-adrenergic receptors on smooth muscle cells to inhibit peristalsis and slow digestion so that resources can be focused on the immediate response to the source of stress.

4.2.4 Electrical and synaptic signalling

In Section 2.8, you learnt that cells are able to maintain an electrical potential, the membrane potential. This arises because the distribution of ions differs on either side of the cell membrane. You also learnt that excitable cells, such as neurons, can generate action potentials that rapidly propagate along the length of the nerve axon. The arrival of the action potential at the synapse of a signalling neuron (Figure 4.2b (iv)) causes the release of a chemical known as a **neurotransmitter** from the axon terminal of the signalling cell (known as the *pre*synaptic neuron). The neurotransmitter diffuses across the space between the two cells (the synaptic cleft, a distance of about 20 nm), and binds to a specific receptor on the target cell (the *post*synaptic neuron). Binding of the neurotransmitter to the receptor either elicits a direct response in the target cell, such as opening receptor-operated ion channels (Section 4.5) that generate an electrical signal, or activates other proteins that initiate a series of intracellular responses within the target cell (Sections 4.4 and 4.6).

A variety of neurotransmitters are used by different types of neuron to signal to their target cell across a synapse. Most are small molecules such as the amino acid neurotransmitters (e.g. glutamate), modified amino acids (e.g. dopamine and noradrenalin), acetylcholine, and even nucleotides (e.g. ATP). Some larger molecules such as small polypeptides (e.g. endorphins) are also released by neurons and act as neurotransmitters.

Electrical signalling also occurs in plants to coordinate activity. An example of activity coordinated by electrical signalling in a plant is the capture of an insect by the Venus fly-trap, which uses insects as a source of minerals. The trap consists of a modified leaf with two lobes either side of the mid-rib, which acts as a hinge. Each of the two lobes carries long, stiff projections at their margin to aid in capturing the insect, and three trigger 'hairs' near the hinge. These trigger hairs act as an external sensor, and multiple touches of the trigger hairs are required to generate a signal (either two hairs need to be touched, or one hair twice in quick succession). When an insect lands on the leaf, it moves the trigger hairs and an action potential is generated in a sensory cell at the base of the hair. If the movement of the fly triggers action potentials in both lobes of the trap, they close together and trap the insect, which is then digested by secreted extracellular enzymes.

4.2.5 What makes a good intercellular signalling molecule?

Many different types of molecule act as intercellular messengers, but they generally have some common characteristics. The majority are small, and so readily diffuse or are carried from the site of synthesis to the site of action. Some examples of the different types of molecules that act as intercellular signals in mammals are shown in Table 4.1.

Table 4.1 Examples (not exhaustive) of molecules used in signalling between mammalian cells.

Intercellular signalling molecules	Type of molecule	Examples of actions	Type of signalling
oestrogen, testosterone	steroids	control of reproductive function	endocrine
prostaglandins	fatty acid-derived (eicosanoids)	smooth muscle contraction (e.g. uterus); platelet aggregation (at sites of vascular injury)	autocrine
nitric oxide	small inorganic molecule (dissolved gas)	mediator of smooth muscle relaxation and of some interneuronal signalling	neuronal and paracrine
endorphins	peptides	interneuronal signalling (several functions including involvement in perception of pain)	neuronal
insulin	small protein	regulation of glucose metabolism	endocrine
cytokines, e.g. interleukins	small proteins	local cell responses, e.g. immune system cells; stimulate growth and division of cells	paracrine (some autocrine)
growth factors, e.g. epidermal growth factor (EGF)	small proteins	stimulate cell growth, division, differentiation and/or survival	paracrine
glutamate	amino acid	signalling between some neurons in the brain	neuronal
acetylcholine	small organic molecule (a choline ester)	signalling in the autonomic and peripheral nervous systems and the brain; triggers contraction of skeletal muscles.	neuronal
adrenalin	small organic molecule (a catecholamine)	regulating heart rate, circulation and breathing; triggers the 'fight or flight' response of the sympathetic nervous system	endocrine and neuronal

A further characteristic of a signalling molecule is that it can be immediately secreted on demand. This property is important, since cellular responses often need to be very rapid. In many cases, ready availability is achieved by synthesising signalling molecules in advance, which are then stored in vesicles near their site of release. Such is the case with many neurotransmitters: for example, acetylcholine which is used by mammals to cause the contraction of skeletal muscles.

Finally, the speed and efficiency of removal of a signalling molecule after it has been released is also important for many types of signalling.

■ Why would it be important that a signal is removed rapidly and efficiently once it has reached the target cell?

□ Once a signal has been produced and the appropriate response has been elicited, the signal must be 'turned off' to prevent an excessive response by the target cell.

After release, intercellular signalling molecules may be eliminated by degradation (by extracellular enzymes or as a result of intrinsic chemical instability), or by being taken up inside the signalling cell, the target cell or neighbouring cells. The significance of the latter mechanism for controlling responses to extracellular signals is apparent from the clinical applications of drugs that affect the reuptake of signalling molecules. Examples include those that inhibit the cellular uptake of the neurotransmitter serotonin; such drugs, which effectively increase extracellular levels of serotonin and prolong the exposure of the target cell to this signal, are used to treat depression in humans.

Summary of Section 4.2

- Adjacent cells can communicate by direct physical contact between signalling and receptor molecules that are anchored or located in the cell membranes, or can use gap junctions to exchange signalling molecules.
- Other cells release signalling molecules that act locally on nearby cells (paracrine signalling) and sometimes on themselves (autocrine signalling).
- Endocrine signalling in animals involves the release of signalling molecules into the bloodstream where they are then transported to their target cells. Glands that employ this mechanism are commonly referred to as endocrine glands.
- Neurons also use signalling molecules; these are released at the synapse in response to electrical activation and act on the downstream neuron by binding to receptors located on it.
- Intercellular messengers are generally small, secreted on demand and are efficiently removed to 'turn off' the signal.

4.3 Signal reception

Having considered the signals that are the means by which cells communicate with each other, this section will look at how these signals are received by the target cell. As you already know, there are two main types of signalling molecule: those, such as steroid hormones, that can cross the cell membrane, and those that cannot cross the cell membrane but act via receptors on the cell surface (introduced in Chapter 2).

4.3.1 Small inorganic signalling molecules do not act via cell surface receptors

The small inorganic molecules nitric oxide (NO) and carbon monoxide (CO) act as signalling molecules. They can easily pass through cell membranes and do not require a receptor to relay their signals.

- ■ Recalling from Section 2.7, why is it that NO and CO can cross the cell membrane?
- □ As small, gaseous uncharged molecules, NO and CO can readily diffuse across the lipid bilayer of the cell membrane.

For many years it was known that relaxation of vertebrate blood vessels (also known as vasodilation) was brought about by a factor produced by the endothelial cells that line the inner surface of the blood vessels. Originally known as endothelium-derived relaxing factor (EDRF), in 1987 this factor was demonstrated to be the dissolved gas NO. Since then, much research has led to the elucidation of the mechanism of NO function.

Endogenous NO is produced by endothelial cells in response to the neurotransmitter acetylcholine, which is released from stimulated nerve endings that terminate on the blood vessels. NO causes the smooth muscle in the walls of the blood vessels to relax, which increases the diameter of the blood vessels, allowing more blood to flow through them. Identification of the mode of action of NO on blood vessels has provided an explanation of how nitroglycerine, a common treatment for the heart complaint angina, functions. Angina is a condition that occurs when the coronary arteries (those that supply the heart muscle itself with blood) become partially blocked and the heart muscle does not receive enough oxygen to pump blood efficiently. Angina causes severe pain that is very rapidly alleviated by taking the water-soluble drug nitroglycerine. Once dissolved, nitroglycerine is rapidly converted in the body into NO, causing dilation of the blood vessels. The heart has to do less work to pump blood through dilated vessels, thus reducing the oxygen requirement of the heart muscle. NO is also utilised as a signalling molecule by the cells of the immune system, and, in the nervous system, it is an important signal used by neurons.

How does NO exert its effect? NO is formed from the amino acid arginine by the action of the enzyme *nitric oxide synthase* (NOS) (Figure 4.4). Once the NO is released from a cell (e.g. an endothelial cell), its half-life (the time for half of the NO molecules to be metabolised) is very short, of the order of 5–10 seconds; so the effects of NO are usually quite local despite the fact that NO is soluble and can readily pass through cell membranes. Once inside the target cell, NO binds to and activates the enzyme **guanylate cyclase**, which catalyses the production of **cyclic GMP (cGMP)** from GTP. cGMP acts as a 'second messenger', and transfers the signal onwards within the cell where it activates the cellular response.

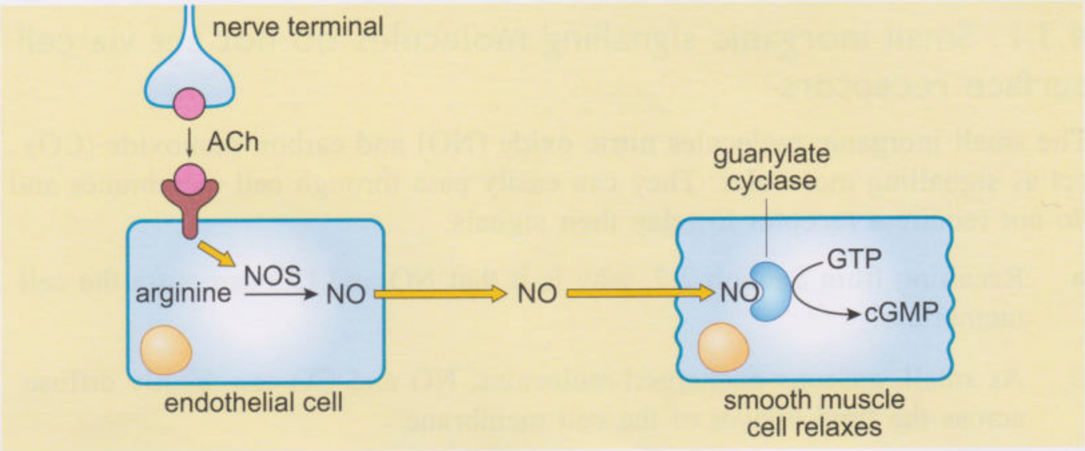


Figure 4.4 The action of nitric oxide as a signalling molecule causing relaxation of smooth muscle cells. Endothelial cells release NO in response to stimulation by the neurotransmitter acetylcholine (ACh). The NO diffuses to adjacent smooth muscle cells, where it activates guanylate cyclase to produce cyclic GMP (cGMP), which causes the cell to relax.

4.3.2 Steroid hormones signal via intracellular receptors

The steroid hormones, such as the sex hormones oestrogen and testosterone, and the thyroid hormones (which regulate metabolic rate) are molecules that, due to their hydrophobic nature, can pass directly through the cell membrane. Once inside the cell, they bind to intracellular receptors and ultimately affect the rate of transcription of particular target genes. The intracellular steroid receptors belong to the steroid hormone receptor superfamily and fall into two categories depending on whether they are present in the cytosol (cytosolic intracellular receptors) or only found in the nucleus (nuclear intracellular receptors).

The cytosolic intracellular receptors are maintained in an inactive form by association with inhibitory proteins; but on binding of the appropriate steroid hormone they undergo a conformational change, which causes the inhibitory proteins to dissociate from them. A 'freed' cytosolic receptor, along with its bound steroid hormone ligand, can enter the nucleus where it acts as a transcription factor, binding directly to specific regulatory sequences on the DNA to activate or repress transcription of particular target genes (Book 1, Section 6.4). The regulatory DNA sequences to which the receptor–hormone complexes bind are known as hormone response elements (HREs, see Figure 4.5a).

Nuclear intracellular receptors, as shown in Figure 4.5b, spend most of their time associated with the HRE, but remain in an inactive state until the steroid ligand binds. Once again, binding of the hormone leads to a conformational change resulting in the modification of gene transcription.

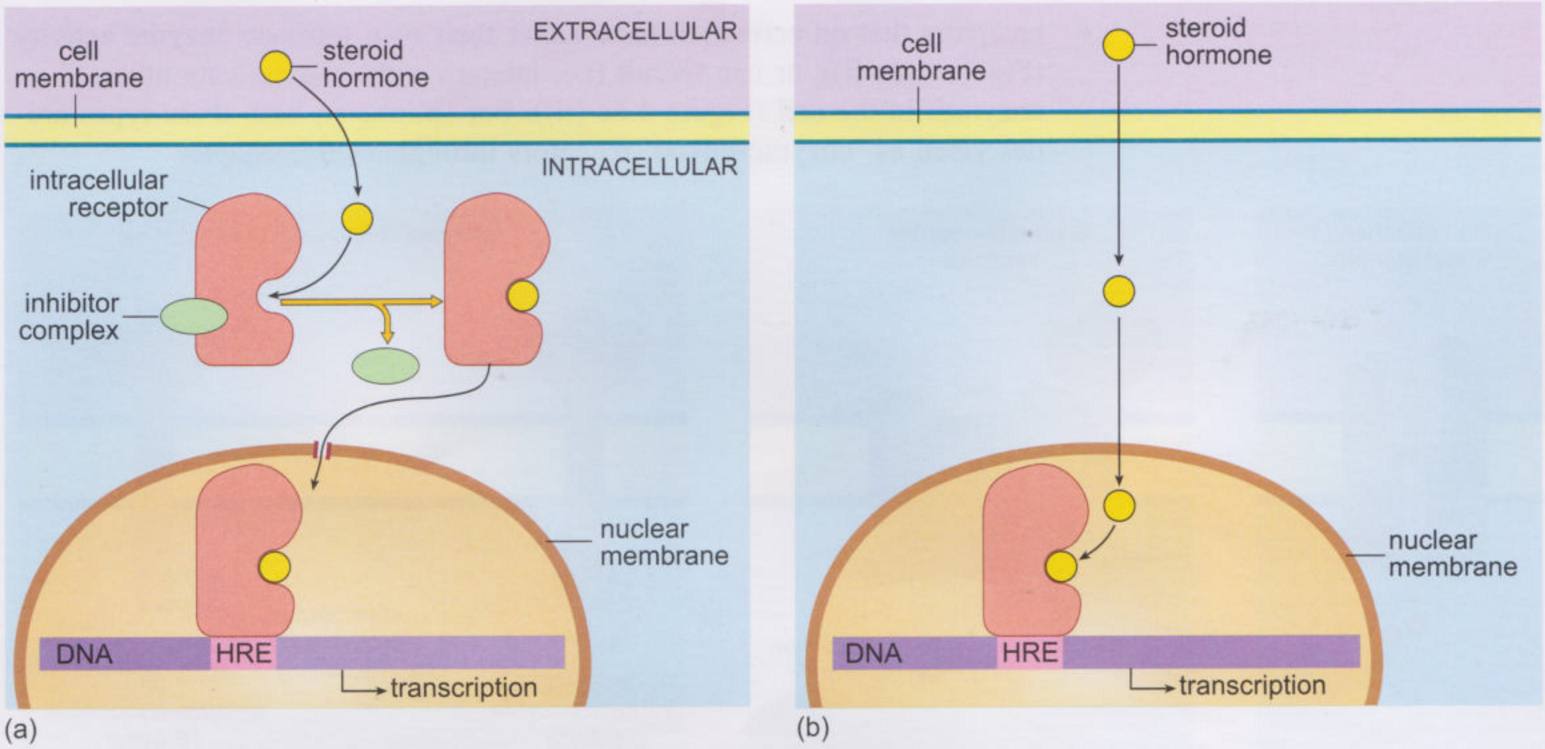


Figure 4.5 Mechanism of steroid activation of gene transcription. (a) Binding of ligands to cytosolic intracellular receptors releases them from inhibitor protein. The free hormone–receptor complex then enters the nucleus and binds to hormone response elements (HREs) on the DNA to activate/repress transcription. (b) Ligands bind directly to nuclear receptors that are already associated with the HRE and so activate/repress gene transcription.

4.3.3 The majority of signalling molecules act via cell surface receptors

Signalling molecules that cannot cross the cell membrane mediate cell communication via cell surface receptors. These receptors are transmembrane proteins (described in Section 2.4.2) that undergo a conformational change when they bind a ligand. This is the first stage in the transduction of the signal.

■ What are the key structural features of transmembrane proteins?

□ Transmembrane proteins span the cell membrane, having an extracellular domain, at least one hydrophobic membrane-spanning domain and an intracellular (cytosolic) domain.

In the case of cell membrane receptors, it is the extracellular portion of the receptor that binds the signalling molecule, and typically the intracellular domain is critical for signal transduction, often undergoing conformational change.

Cell surface receptors can be classified broadly into one of three main categories, according to the mechanism by which they transduce the signal:

- ion channel-linked receptors (Figure 4.6a)
- G protein-coupled receptors (also known as 7-helix transmembrane receptors; Section 2.4.2) (Figure 4.6b)

- receptors that on activation have either their own intrinsic enzyme activity (Figure 4.6c (i)), or can recruit (i.e. interact with) and activate other enzymes in the cell (Figure 4.6c (ii)). For simplicity, both these types are described as 'enzyme-linked' receptors throughout this chapter.

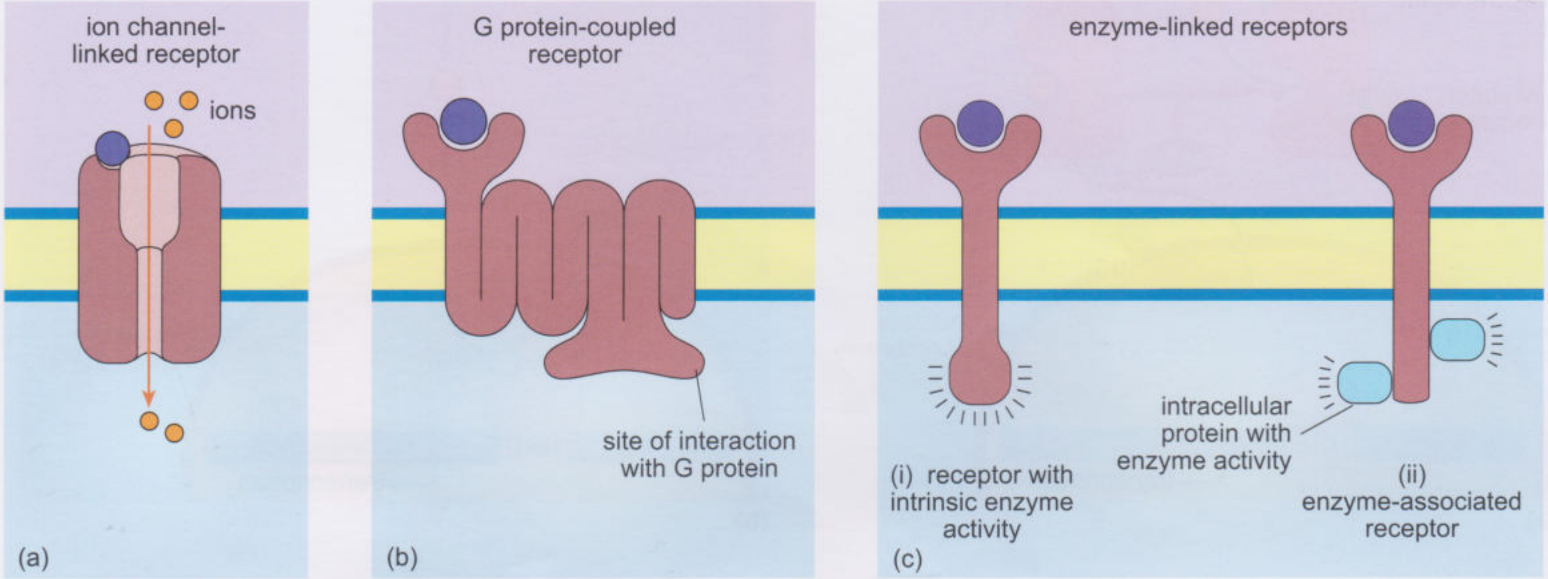

ion channel-linked receptor
ions
(a)
G protein-coupled receptor
site of interaction with G protein
(b)
enzyme-linked receptors
intracellular protein with enzyme activity
(i) receptor with intrinsic enzyme activity
(ii) enzyme-associated receptor
(c)

Figure 4.6 The three major classes of membrane receptor: (a) ion channel-linked receptor; (b) G protein-coupled receptor (7-helix transmembrane receptor); (c) enzyme-linked receptor of which there are two types: (i) receptors with intrinsic enzyme activity, and (ii) enzyme-associated receptors.

Ion channel-linked receptors transduce an extracellular signal into the opening or closing of an ion channel in the cell membrane. Found in neurons and other electrically excitable cells such as muscle cells, they transduce a chemical signal directly into an electrical signal by altering the membrane potential of the target cell. Being responsible for detecting signals from neurotransmitters (the signalling molecules released by neurons), these receptors are sometimes referred to as transmitter-gated ion channels (Section 2.8.1). Binding of the signal leads to a conformational change in the ion channel-linked receptor, which either opens or closes the associated ion channel. The change in ion permeability is very rapid and also very transient as the ion channel reverts rapidly to its normal state soon after ligand binding.

G protein-coupled receptors (GPCRs) operate in a very different way from the ion channel-linked receptors and are more widely used, by both excitable and non-excitable cells. When the ligand binds to the extracellular region of the receptor protein, changes to the cytosolic domain occur that cause the activation of an associated heterotrimeric protein (the G protein), initiating a series of chemical events in the cell that culminates in the cellular response.

■ What is the subunit composition of a heterotrimeric protein?

□ It has three subunits, all different from each other.

Enzyme-linked receptors operate by regulating a specific enzyme activity in the cell. Some, when activated, have their own intrinsic kinase activity and affect the activity of particular enzymes and other proteins in the cell directly by phosphorylating them; others recruit and activate other enzymes. In either case, the targets that are activated as a result of receptor activation in turn regulate the activity of other intracellular signalling molecules.

The following sections focus on what happens when a cell surface receptor is activated, i.e. the intracellular signal transduction mechanisms associated with the different kinds of cell surface receptors.

Summary of Section 4.3

- Some signalling molecules, such as the steroid hormones and nitric oxide, are capable of passing through the cell membrane and bind to receptors or other targets inside the cell.
- Most signalling molecules bind to the extracellular domain of transmembrane receptors located at the cell surface. There are three broad categories of cell surface receptors: ion channel-linked receptors, G protein-coupled receptors and enzyme-linked receptors.

4.4 Features and mechanisms of intracellular signal transduction pathways

You will already have realised that any cell in a multicellular organism (or any individual unicellular organism) is, at any moment in time, exposed to a variety of molecules that may be able to evoke a cellular response. As noted in Section 4.1, while there are many different intercellular signals and receptors that recognise these signals, the intracellular signal transduction mechanisms that transmit the signal onward from the receptor and elicit the response in the cell are *relatively* limited in variety. As will become clear later in this chapter, the diversity and subtlety of responses to signals that are evident in different cell types derive from the complex interactions that occur *between* the intracellular signalling pathways.

The remainder of this chapter outlines the major transduction processes and, where possible, uses as examples those pathways that are triggered by some of the signalling molecules mentioned in Section 4.2. Ion channel-linked receptors provide the simplest mechanism of signal transduction (described in Section 4.5), where binding of the ligand changes the conformation of the receptor and results in opening or closing of ion channels in the cell membrane. The intracellular signalling pathways associated with G protein-coupled receptors (Section 4.6) and enzyme-linked receptors (Section 4.7) are, however, far more complex. The signal received when the ligand binds to this type of receptor is transmitted via a number of signal transduction molecules to the site of response in the cell, which may be in another part of the cell. For example, signal transduction may ultimately bring about changes in expression of a gene by affecting its transcription in the nucleus. The importance of interactions between, and integration of, signalling pathways is considered in Section 4.8.

Whatever the signal or the type of receptor, the sequence of molecular events that follows receptor activation, referred to as a *signal transduction pathway*, is rarely a simple chain, but is instead a branching network of activation (and deactivation) of signalling molecules. Signal transduction pathways are commonly described as *cascades* as, at each stage, both the number of types of molecule and the numbers of molecules involved increases (Figure 4.7).

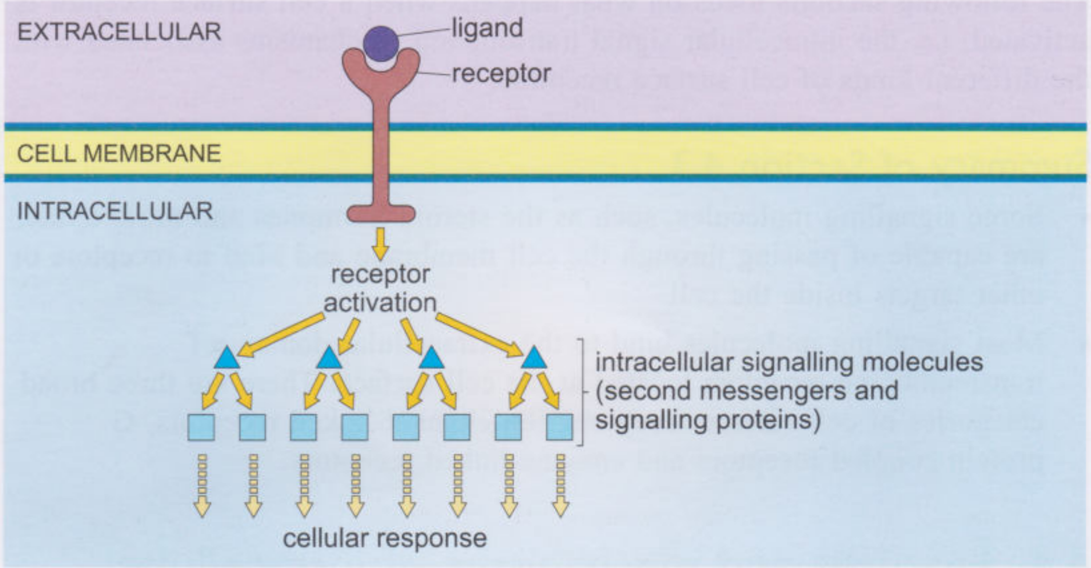


Figure 4.7 Schematic diagram showing the main principles that underlie signal transduction after activation of G protein-coupled and enzyme-linked receptors. Binding of the ligand activates the receptor, which transduces the signal to the intracellular compartment. The initial signal is amplified by recruitment or generation of increasing numbers of intracellular signalling molecules (second messengers and signalling proteins) in a chain of reactions within the cell, resulting in a cascade effect. The signal is distributed in the cell and ultimately transferred to the site(s) of the cellular response(s).

The increased number of signalling molecules ensures that there is a huge amplification of the initial signal, a critical factor in the success of signal transduction. Furthermore, with increasing numbers of different types of intracellular signalling molecule being recruited, activated or generated at each stage, there is also, most importantly, scope for integration with other pathways and diversification and modulation of responses.

Intracellular signalling molecules are of two main types: second messengers and signalling proteins. Second messengers are small readily diffusible intracellular mediators whose concentration changes rapidly on receptor activation, either through *de novo* synthesis as in the case of cAMP (Section 4.6.2) or by release from intracellular stores as in the case of calcium ions (Section 4.6.3). While the term 'second messenger' is widely used, it is rather misleading, as these intracellular signalling molecules are not necessarily second to the receptor activation event, but may in fact be quite far down the cascade. Signalling proteins generally, but not exclusively, function by altering the activity of the next signalling protein in the cascade, or by modifying the concentration of second messengers. Note that some intracellular signalling molecules are common to a number of different signalling cascades, and this is one way in which 'cross-talk' between signalling pathways can occur.

There are a number of general mechanisms by which a signal is transferred from one molecule to another. The fundamental principle that underlies all these mechanisms is one that you are already familiar with: a change in protein conformation that can bring about a change in the protein's activity. Changes in conformation can be brought about by binding of small molecules or ions or by chemical modification of the protein by the action of an enzyme. Some of the most common general mechanisms that are used to activate proteins involved in signal transduction pathways are summarised below:

1 Phosphorylation of specific amino acid residues (tyrosine, serine or threonine) on proteins, catalysed by specific kinase enzymes. Note that many proteins have a number of sites that can be phosphorylated and different kinases phosphorylate different sites. Depending on the protein and the residue that is phosphorylated, phosphorylation may activate or inactivate a protein.

■ What do the amino acids tyrosine, serine and threonine (see Table 1.1) have in common that distinguishes them from the other amino acids that are found in proteins?

□ They all have a hydroxyl group (–OH) in their side chains. This is where the phosphate group is added when these residues are phosphorylated.

■ What is one implication for cell signalling mechanisms of the existence of multiple phosphorylation sites on a protein?

□ The protein may need more than one signal to be activated or inactivated.

2 Activation by binding of nucleotides. A number of proteins are activated when bound to specific nucleotides. An example of such a protein is Ras (Section 4.7.1), which is active when bound to GTP (guanosine triphosphate).

3 Activation of enzymes by calcium binding or by calcium-binding proteins.

4 Activation of proteins by small lipid second messengers.

5 Formation of protein complexes with *adaptor proteins*, which link receptors to other signalling molecules.

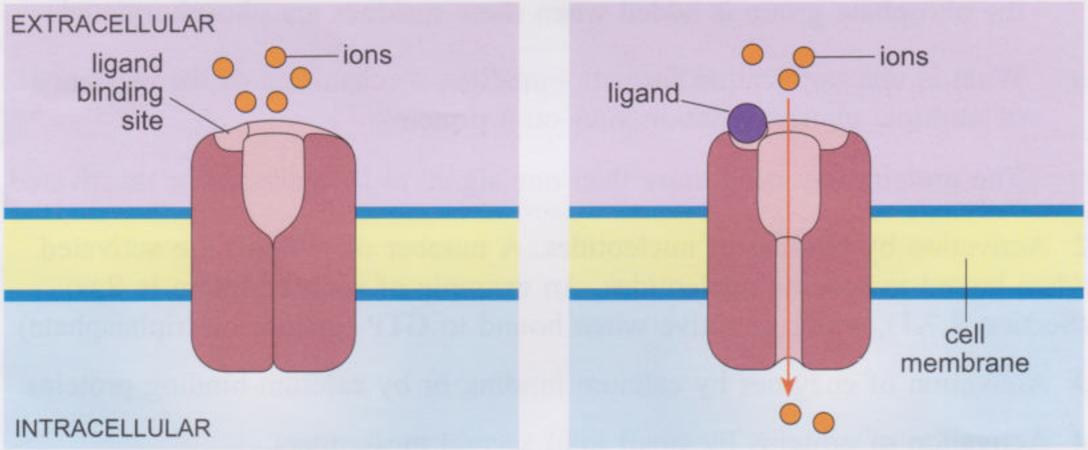
Summary of Section 4.4

- The binding of a ligand to its receptor causes a conformational change in the protein receptor which initiates intracellular signalling.
- Signal transduction pathways amplify the signal and allow integration with other signalling pathways.
- Binding of a ligand to an ion channel-linked receptor causes the channel to open or close. The intracellular signalling cascades associated with G protein-coupled and enzyme-linked receptors are more complex, involving various second messengers and signalling proteins.
- Proteins in a signalling cascade or pathway are activated or inactivated by either phosphorylation, binding of nucleotides (e.g. GTP), calcium binding or calcium-binding proteins, small lipid second messengers, or the formation of protein complexes with adaptor proteins.

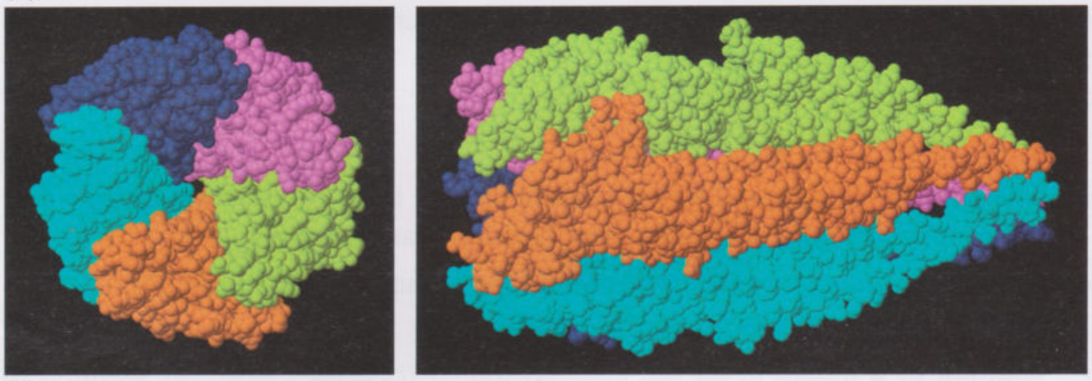
4.5 Signal transduction by ion channel-linked receptors

Signal transduction by ion channel-linked receptors is relatively simple. When the ligand binds to the receptor on the extracellular surface, the receptor conformation changes, which has the effect of opening or closing an ion channel in the cell membrane (Figure 4.8a). By opening or closing the channel, the receptor controls the movement of specific ions (e.g. Na^+, K^+, Cl^- or Ca^{2+}) across the cell membrane. The ions move in the direction dictated by their electrochemical gradients (Section 2.8.3).

Ion channel-linked receptors are generally composed of a number of membrane-spanning subunits that are arranged in a ring, forming a narrow channel or pore. Once the ion channel is open, ions can move into or out of the cell down their electrochemical gradient. These channels are fast-acting and are usually specific for one or two types of ion.



(a)



(b)

Figure 4.8 (a) Ion channel-linked receptors have a central channel. In this illustration, the channel remains closed in the absence of ligand but when the ligand binds to the receptor, it causes a conformational change which opens the channel. While the channel is open, ions of a particular type are free to cross the membrane in the direction dictated by their electrochemical gradient (in this illustration, passing into the cell). (b) Structure of the nicotinic acetylcholine receptor from the marbled electric torpedo ray (*Torpedo marmorata*; Protein Data Bank entry 2bg9), shown in top-down and side view in the membrane. The five subunits are coloured differently.

- ■ What kind of amino acid side chains would you expect to predominate in the polypeptides that form the pore-facing surface of the channel?
- □ Hydrophilic amino acid side chains predominate, creating a hydrophilic environment through which the charged ions can cross the hydrophobic interior of the cell membrane.

Perhaps the best-understood example of an ion channel-linked receptor is one that binds the neurotransmitter acetylcholine (Table 4.1), the nicotinic acetylcholine receptor. This receptor is expressed in mammals on some neurons and on skeletal muscle cells and, as well as binding acetylcholine, can bind nicotine (from the tobacco plant), hence its name. Figure 4.8b shows the structure of the nicotinic acetylcholine receptor from the marbled electric torpedo ray (*Torpedo marmorata*), which is similar to that found on human skeletal muscle cells at the site of nerve–muscle synapses (also known as neuromuscular junctions). In the torpedo ray, this ion channel-linked receptor is present in very high numbers in the electric organ of the ray, which it uses to shock and stun prey or predators.

The nicotinic acetylcholine receptor is composed of five subunits that span the cell membrane and the top-down view shows a narrow channel that extends through the membrane. Two of the subunits have binding sites for acetylcholine and, when the neurotransmitter binds, the shape of the entire receptor changes slightly, opening up the channel such that Na^+ and K^+ can cross the membrane. When nicotinic acetylcholine receptors on skeletal muscle fibres are activated, an action potential (Section 2.8.4) spreads along the muscle fibre, causing muscle contraction. (The mechanism of contraction of skeletal muscle is discussed in Section 5.3.)

Summary of Section 4.5

- When a signalling molecule binds to an ion channel-linked receptor, the receptor conformation changes, causing the channel to open or close.
- Ions move across the cell membrane in the direction dictated by their electrochemical gradients. The ion channels are usually specific for one or two types of ion.

4.6 Signal transduction by G protein-coupled receptors

The G protein-coupled receptors (GPCRs) represent the largest family of cell surface receptors. Humans have more than 700 such different receptors and the genes that encode them represent approximately 4% of the protein-coding part of the human genome. A variety of different intercellular signalling molecules are involved in the signalling pathways that start with activation of GPCRs. In all cases, the initial events that follow GPCR activation involve intracellular proteins that are known as G proteins (so called because they bind the nucleotide GTP), which are located on the intracellular side of the cell membrane.

GPCRs are formed from a single polypeptide chain which crosses the membrane seven times (Figure 4.6b). The seven membrane-spanning stretches of the protein are α-helices; thus these receptors are often called the 7-helix transmembrane receptors. The extracellular N-terminus of the protein is the site of interaction with the ligand and the cytosolic loop linking the fifth and sixth transmembrane helices is the region that interacts with the G proteins.

4.6.1 G protein activation

To understand the mechanism of signal transduction by G proteins, you need to know something about their structure. G proteins are heterotrimeric; that is, they are made up of three different protein subunits, known as the α, β and γ subunits. The α and γ subunits both have a lipid anchor, which tethers them at the cytosolic surface of the cell membrane. The β subunit is not anchored to the membrane but is tightly associated with the γ subunit (Figure 4.9a). When a G protein is inactive, i.e. when its receptor is not bound to its ligand, the α subunit is associated with the nucleotide GDP (guanosine diphosphate) and with the βγ subunit complex, as shown in Figure 4.9a.

Upon binding its ligand, a GPCR undergoes a conformational change and binds the inactive GDP-bound G protein (Figure 4.9b), which triggers the release of GDP and the binding of GTP to the α subunit of the G protein (Figure 4.9c). GTP binding triggers the activation of the α subunit (again through a conformational change) and it dissociates from the βγ subunit complex (which in some cases also becomes activated, Figure 4.9c). The activated α subunit and βγ complex can both move freely along the cell membrane and interact with target proteins located nearby, further transducing the signal (Figure 4.9d). The subunits will remain active until the GTP bound to the α subunit is hydrolysed to GDP, a reaction that occurs quite rapidly because the α subunit itself has intrinsic GTPase activity (the ability to hydrolyse GTP to GDP, Figure 4.9e). Once the GTP is hydrolysed to GDP, the α subunit reunites with the βγ complex, thereby inactivating both of these components (Figure 4.9a).

Cells contain a number of different types of G protein. Most variation has been found in the α subunit, with different forms interacting with different protein targets. In some cases, the α subunit may be stimulatory, activating its target protein, while others are inhibitory, inhibiting the activity of its target protein. Moreover, a single type of activated subunit may act on more than one downstream target. The target proteins of activated G protein subunits are either ion channels (Figure 4.10b (i)) or membrane-bound enzymes. Thus the molecular signal is transduced either into an electrical signal by the opening or closing of ion channels, or into a signalling cascade involving other intracellular signalling molecules.

An overview of some of the targets of activated G proteins is shown in Figure 4.10. Note that there are numerous other targets of activated G proteins, but only a few examples are described here. The major membrane-bound enzyme targets of activated G proteins are **adenylate cyclase** and **phospholipase C (PLC)** (Figure 4.10b (ii) and (iii)) and the next section describes the signal transduction pathways triggered by activation of these two enzymes by G proteins.

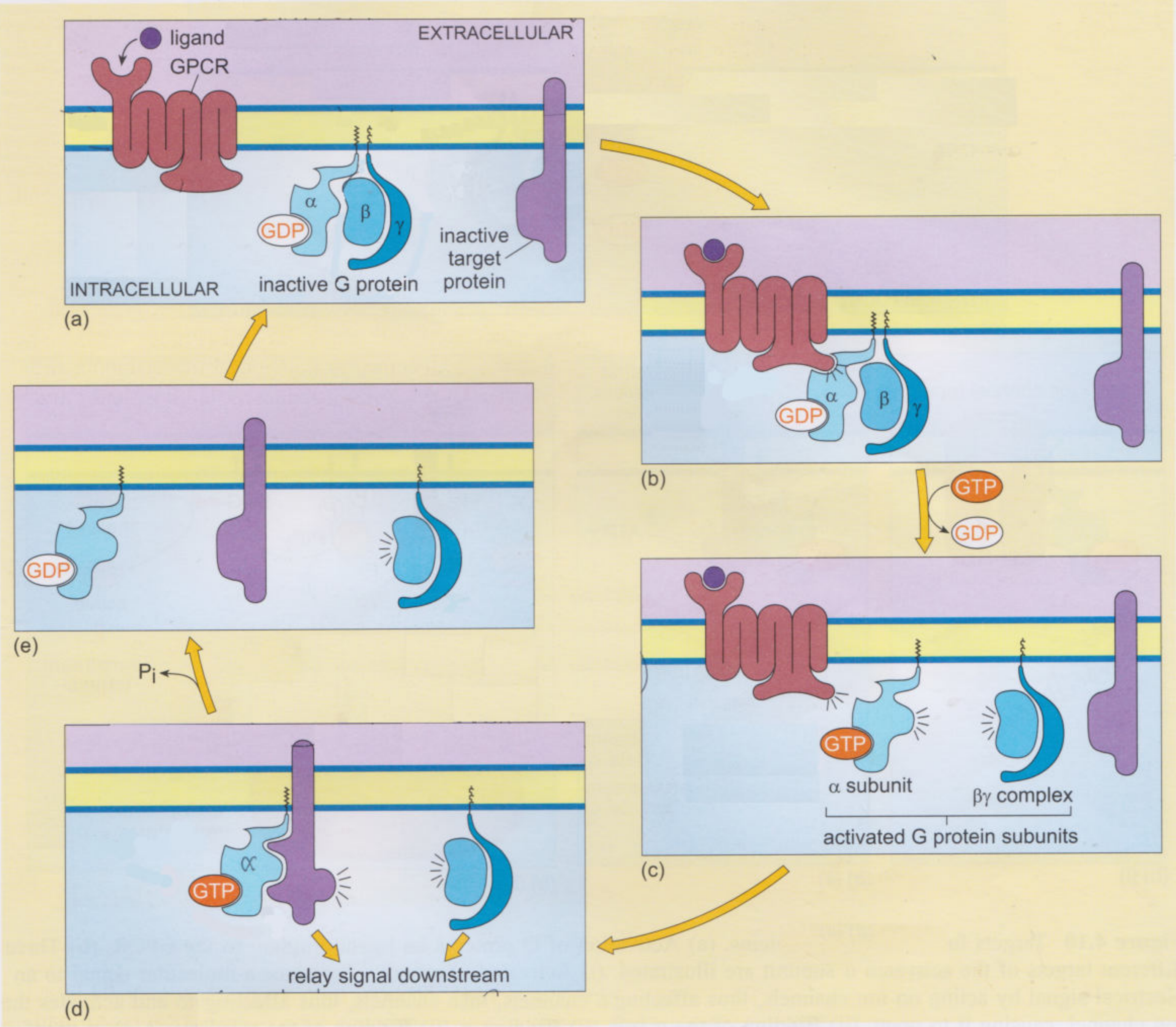


Figure 4.9 Signalling through G protein-coupled receptors (GPCRs). (a) All components of the signalling pathway are shown in their inactive form. (b) The change in conformation of the GPCR on ligand binding brings about the binding of the trimeric G protein. (c) GTP displaces GDP on the α subunit thereby activating it and causing it to dissociate from the βγ complex. (d) The α subunit binds and activates target proteins, which also act as effectors (intracellular signalling molecules) and propagate the signal. Note that in some cases the βγ complex activates other pathways downstream. (e) Inactivation of the α subunit via GTPase activity (whether intrinsic or accessory, i.e. involving a separate enzyme) results in dissociation from the target protein (which itself becomes inactivated) and re-formation of the inactive trimeric G protein complex by association with a βγ complex.

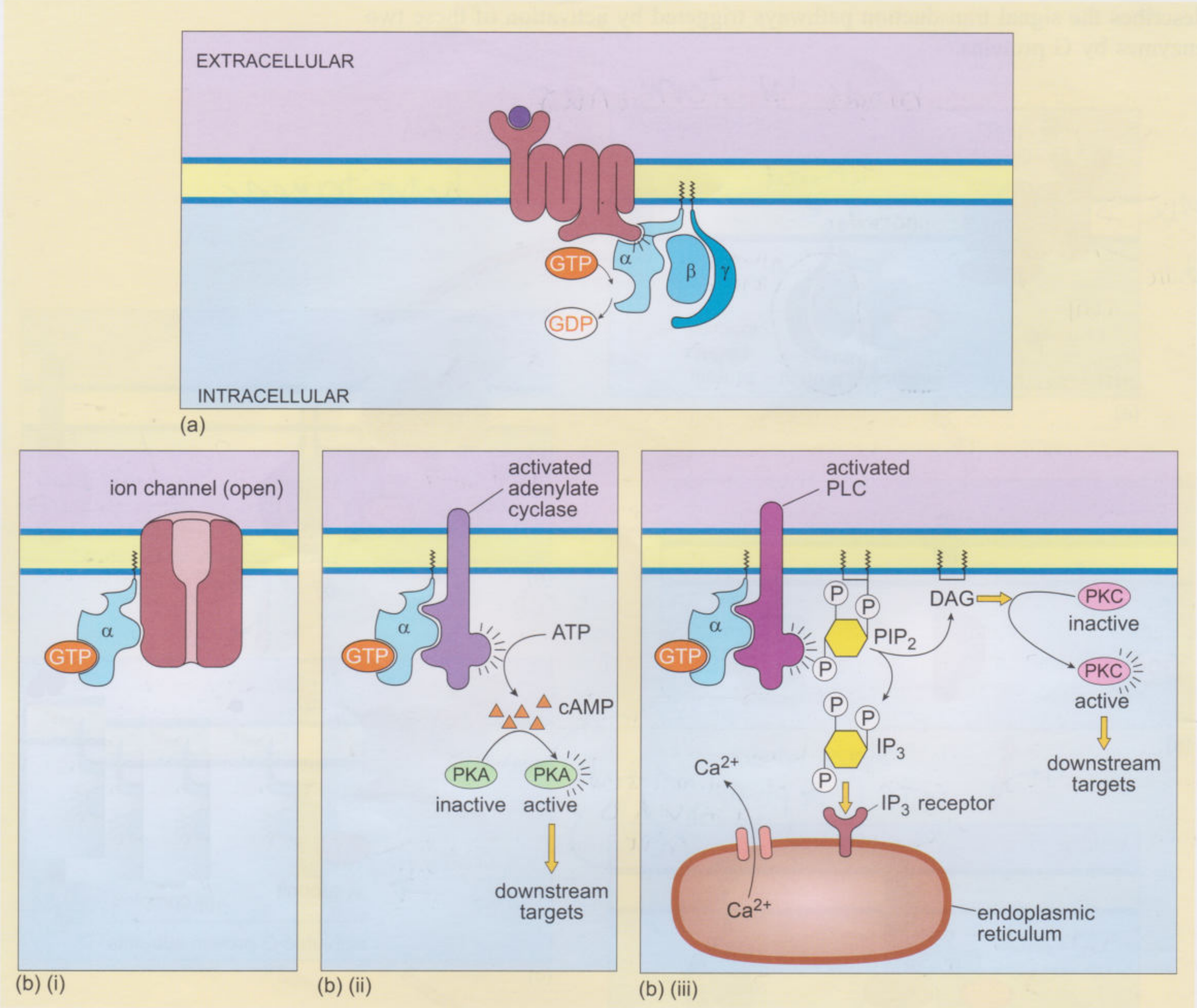


Figure 4.10 Targets for activated G proteins. (a) Activation of G proteins on ligand binding to the GPCR. (b) Three different targets of the activated α subunit are illustrated. (i) Activated G proteins transduce a molecular signal to an electrical signal by acting on ion channels, thus affecting membrane potential; the α subunit binds to and activates the ion channel, causing it to open. (ii) Binding of the α subunit leads to activation of adenylate cyclase. Activation of adenylate cyclase leads to the production of cyclic AMP (cAMP), which in turn activates cytosolic protein kinase A (PKA). Activated PKA goes on to phosphorylate a number of downstream targets leading, eventually, to a cellular response. (iii) Schematic diagram of inositol phospholipid signalling. The activated α subunit of the G protein activates phospholipase C (PLC), which hydrolyses the inositol phospholipid PIP_2 into two second messengers: 1,2-diacylglycerol (DAG), which remains in the membrane, and inositol 1,4,5-triphosphate (IP_3), which diffuses into the cytosol. DAG activates protein kinase C (PKC) which goes on to phosphorylate downstream targets, while IP_3, acting via a receptor, activates calcium channels in the endoplasmic reticulum, leading to an increase in the concentration of calcium in the cytosol. See text for details.

4.6.2 G protein regulation of adenylate cyclase: cAMP as a second messenger

As shown in Figure 4.10b (ii), once activated by G proteins, adenylate cyclase catalyses the formation of the second messenger **cyclic AMP (cAMP)**, from ATP. cAMP diffuses readily within the cytosol and activates the next enzyme in the cascade, known as **PKA (protein kinase A**, or cAMP-dependent protein kinase), which phosphorylates specific serine or threonine residues of downstream protein targets. The downstream proteins that are phosphorylated by PKA vary according to the type of cell that is receiving the signal; thus a single signalling system can produce different responses in different cell types.

A good example of a signalling pathway involving adenylate cyclase activation and the generation of the second messenger cAMP is that triggered by binding of the extracellular signalling molecules noradrenalin and adrenalin to GPCRs known as β-adrenergic receptors (Section 4.2.3). Noradrenalin and adrenalin are released from a group of cells in the adrenal gland at times of stress. In the nervous system, noradrenalin is also released as a neurotransmitter from some neurons. In heart muscle, activation of β-adrenergic receptors by adrenalin leads to effects on ion channels, causing changes in membrane potential that result in an increase in the rate and strength of heart muscle contraction. In contrast, in skeletal muscle the activation of β-adrenergic receptors leads to effects on the activity of the cytosolic enzymes involved in glycogen metabolism. Glycogen is a polysaccharide composed of linked glucose subunits which acts as a major storage material for glucose in animals. At times of stress, extra glucose may be needed by the skeletal muscle (as part of what is known as the 'fight or flight' or acute stress response); so glycogen stored in the muscle is broken down by the action of the enzyme glycogen phosphorylase. This enzyme is *activated* by β-adrenergic receptor activation; whereas the enzyme glycogen synthase, which converts glucose into glycogen, is *inactivated* by adrenergic activation. All these different responses to activation of the β-adrenergic receptor are mediated via the second messenger cAMP, which activates the enzyme PKA, which in turn phosphorylates the various target molecules (Figure 4.11).

In mammals, the adrenal glands are endocrine glands associated with the kidneys, which actually consist of two glands fused together – the adrenal medulla and the adrenal cortex. The adrenal medulla secretes adrenalin and noradrenalin into the circulation in response to acute stress. The adrenal cortex is involved in longer-term stress responses and secretes corticosteroids.

■ What important aspect of protein phosphorylation is illustrated by the differential effects of PKA on the two enzymes involved in glycogen metabolism?

□ Phosphorylation inhibits some enzymes, but activates others, depending on the individual enzyme.

As well as cellular responses that involve activation or inhibition of pre-existing target proteins as described above, cAMP activation can also activate the transcription of particular genes. One of the protein targets of PKA, known as CREB (**c**yclic AMP **r**esponse **e**lement **b**inding protein), once phosphorylated, translocates (moves) to the nucleus, where it binds to specific regulatory sequences on DNA called **cAMP response elements (CRE)**. CREB acts as a transcription factor (Book 1, Chapter 6), activating transcription of specific genes including those that encode enzymes involved in the synthesis

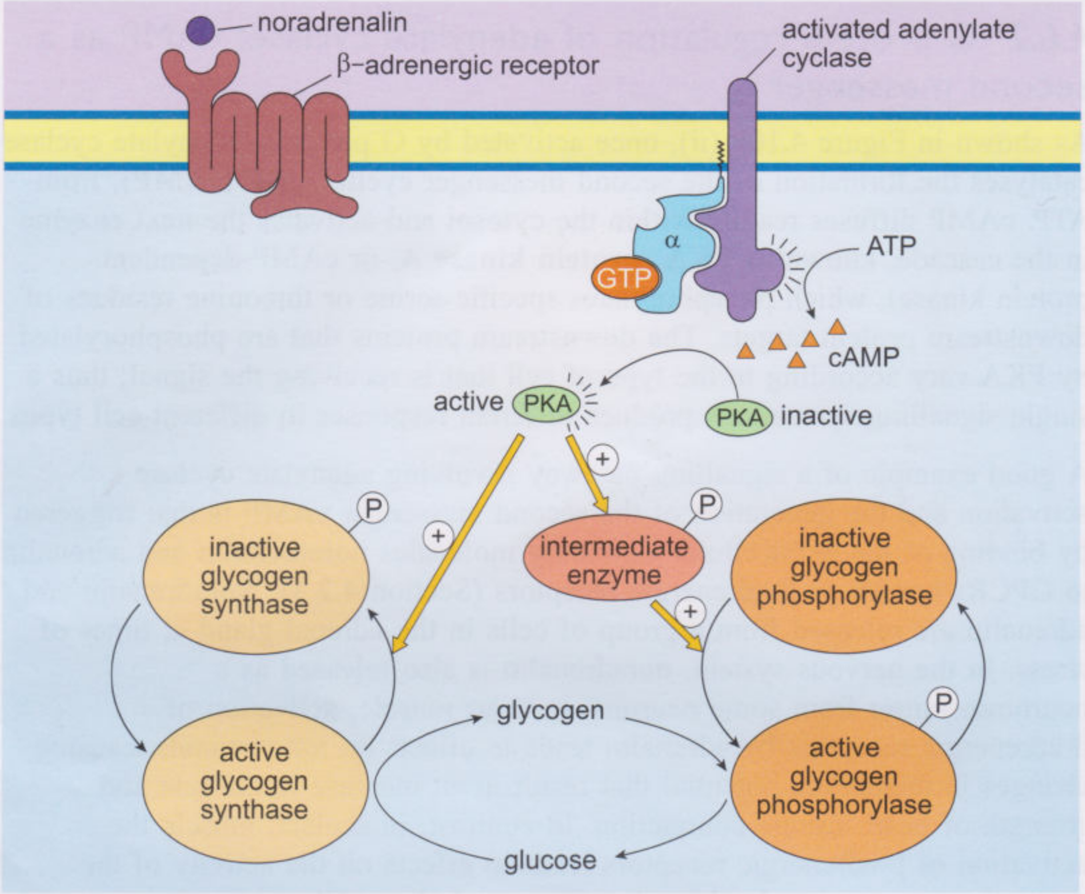


Figure 4.11 The effects of adrenergic activation on the enzymes involved in glycogen synthesis and breakdown. Activation of the β-adrenergic receptor, activation of the α subunit of the associated G protein and the consequent activation of adenylate cyclase stimulates the formation of cAMP, which activates PKA. PKA in turn inactivates glycogen synthase but activates glycogen phosphorylase (by an intermediate enzyme). The end result maximises glucose levels in the circulation.

of glucose in liver cells – yet another means of elevating glucose levels at times of stress.

How is signalling via cAMP terminated? cAMP is rapidly and continuously destroyed in the cell by enzymes known as cAMP phosphodiesterases, which hydrolyse cAMP to adenosine monophosphate (AMP). As with all signalling cascades, efficient signalling requires a fine balance between the activation and removal of the second messenger. The rapid formation and removal of cAMP means that its intracellular concentration can increase or decrease tenfold in a matter of seconds.

The downstream targets of the pathway must also be inactivated by dephosphorylation, carried out by specific protein phosphatases. There are many different types of protein phosphatases that act on specific proteins in the cascade. Some specifically dephosphorylate enzymes or other proteins phosphorylated by PKA, thereby terminating the cascade. Other phosphatases dephosphorylate CREB, thereby terminating the changes in gene activation initiated by receptor stimulation.

4.6.3 G protein regulation of phospholipase C (PLC): IP_3 and DAG as second messengers

The activation of the membrane-bound enzyme phospholipase C (PLC) by activated α subunit results in the generation of two second messengers: the membrane-bound molecule **1,2-diacylglycerol (DAG)** and **inositol 1,4,5-triphosphate (IP_3)**, which is cytosolic. These molecules are both produced when activated PLC catalyses the breakdown of the inositol phospholipid **PIP_2** (phosphatidylinositol 4,5-bisphosphate). In Section 2.4.1, phosphatidylinositols were introduced as one type of phospholipid in which the polar head group consists of a phosphate group to which an inositol sugar is attached. PIP_2 has a further two phosphate groups attached to the inositol ring, as represented in Figure 4.10b (iii). Activated PLC breaks the bond that connects the head group to the glycerol and fatty acyl chains in PIP_2, thus generating a hydrophilic molecule (IP_3) and a hydrophobic molecule (DAG).

As a small polar water-soluble molecule, IP_3 diffuses rapidly through the cytosol, while the larger hydrophobic DAG remains associated with the cell membrane. The downstream events activated by these two second messengers are represented in Figure 4.10b (iii) and are described further below.

- ■ What advantage is conferred by the production of two different second messengers upon activation of PLC?
- □ The production of two second messengers increases the scope for activation of different downstream targets. In addition, the sites of action of the two messengers are different (IP_3 in the cytosol and DAG in the cell membrane), thus increasing the potential range of downstream targets after PLC activation.

Many different cell surface receptors are linked via G proteins to this inositol phospholipid signal transduction pathway, including enzyme-linked receptors for the neurotransmitters acetylcholine (which therefore have both ion channel-linked receptors (Section 4.5) and GPCRs).

IP_3 regulates intracellular Ca^{2+} and activates Ca^{2+}-sensitive enzymes

Once produced, IP_3 diffuses through the cytosol and binds to Ca^{2+} channel-linked IP_3 receptors in the membrane of the endoplasmic reticulum (ER) and opens their Ca^{2+} channels. The concentration of Ca^{2+} in the ER is about 10 000-fold greater than in the cytosol. Because of this large concentration gradient, the opening of the ER Ca^{2+} channels triggers a very rapid increase in the cytosolic Ca^{2+} concentration. The release of Ca^{2+} into the cytosol stimulates further release of Ca^{2+} from the ER by activating other Ca^{2+} channels (a process known as calcium-induced calcium release; see Figure 4.12).

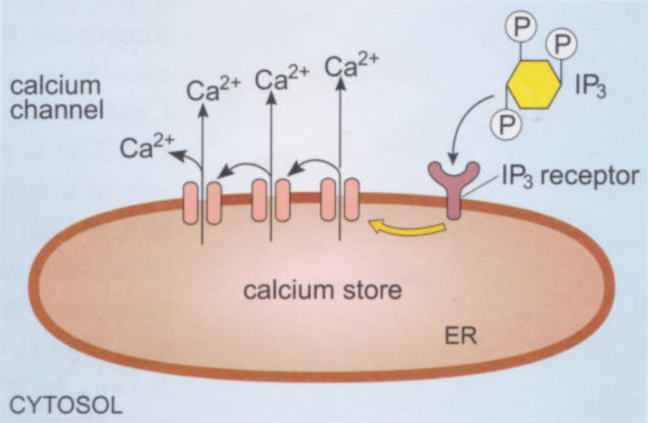


Figure 4.12 Calcium-induced calcium release. Activation of the IP_3 receptors results in release of Ca^{2+} from the ER. The elevated Ca^{2+} levels in the cytosol then stimulate further release of Ca^{2+} from the ER by causing opening of Ca^{2+} channels.

Excitable animal cells such as muscle cells and neurons have a particular type of intracellular Ca^{2+} channel-linked receptor known as ryanodine receptors. These receptors are named after a plant alkaloid to which they are sensitive, and are similar to the IP_3 receptors on the ER. In muscle cells, ryanodine receptors mediate the release of Ca^{2+} from the sarcoplasmic reticulum (smooth ER found in muscle cells), which is an essential step in muscle contraction; while in neurons, they mediate the release of Ca^{2+} from the ER. Ryanodine receptors are activated by changes in cell membrane potential and by increases in cytosolic Ca^{2+}. Thus, as with IP_3 receptors on the ER, they demonstrate calcium-induced calcium release.

Ca^{2+} is a ubiquitous cellular second messenger that has been shown to function in all organisms examined to date, from bacteria to mammals, and is involved in many different signalling pathways.

How does calcium mediate its effects? The sudden increase in the level of Ca^{2+} in the cytosol activates a number of calcium-responsive enzymes. In fact, many of the cellular effects generated by calcium are mediated by another group of intermediate proteins that are activated when Ca^{2+} binds to them. One example is **calmodulin** (often abbreviated to **CaM**), which is present in all eukaryotic cells so far examined. Each calmodulin molecule binds four calcium ions in a cooperative manner; that is, the binding of the first calcium ion facilitates the binding of the next one, and so on. This cooperative binding means that a very small change in the concentration of cytosolic Ca^{2+} can lead to a large change in the activity of calmodulin, a mechanism of propagating and amplifying the signal throughout the cell. On binding calcium ions, calmodulin undergoes a conformational change, which enables it to bind to, and activate, many enzymes. The majority of the effects of calmodulin are via Ca^{2+}/calmodulin-dependent protein kinases (also known as **CaM kinases**, or **CaMKs**), which phosphorylate other target proteins in response to high cytoplasmic Ca^{2+}.

Some of these CaM kinases, such as the CaM kinase in smooth muscle, which transduces the Ca^{2+} signal into cellular contraction, have very narrow specificity, phosphorylating only one type of protein. However, others, such as CaM kinase II (CaMKII), phosphorylate a much broader range of proteins. CaMKII is present in all animal cells, but is particularly abundant in neurons, where it can account for up to 2% of the total cell protein. A particular feature of CaMKII is that it remains active even after the initial calcium signal has been removed from the cell, because once CaMKII has been activated, it phosphorylates itself, a process known as **autophosphorylation**. The activity of CaMKII declines when it is dephosphorylated (by phosphatases), and hence is only terminated when the rate of dephosphorylation exceeds the rate of autophosphorylation.

■ What advantages might autophosphorylation of CAMKII afford a cell?

□ Since autophosphorylation maintains the activity of CaMKII, the cellular effects of the kinase can be prolonged even after the initiating calcium signal is no longer present.

The calcium signal is terminated by two mechanisms: firstly, IP_3 is dephosphorylated and thus inactivated; and secondly, the calcium is removed from the cytosol. Cytosolic Ca^{2+} concentration is kept low in cells by several mechanisms, as shown in Figure 4.13.

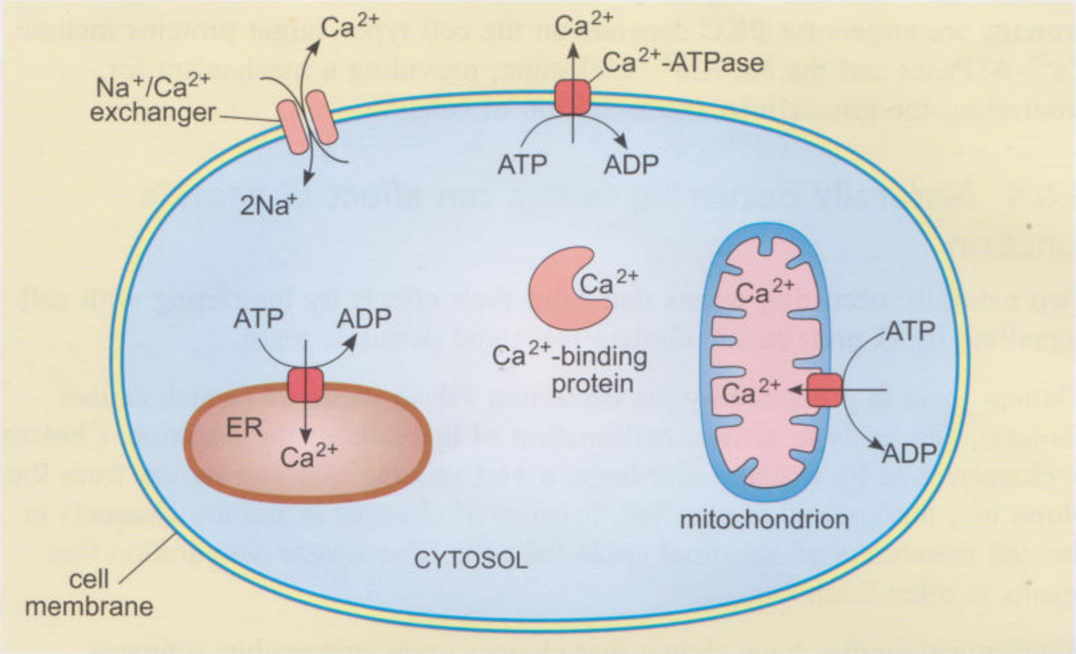


Figure 4.13 Maintenance of low cytosolic calcium concentration by the Ca^{2+}-ATPase pump, the mitochondria and the ER, and calcium-binding proteins. In electrically excitable cells, the Na^+/Ca^{2+} exchanger, located on the cell membrane, also acts to lower cytosolic Ca^{2+} concentrations.

Calcium ions are actively pumped out of all eukaryotic cells by transmembrane Ca^{2+}-ATPases, which use one molecule of cellular ATP to pump out one calcium ion. A similar Ca^{2+}-ATPase is situated in the membrane of the ER, which actively sequesters calcium by taking it out of the cytosol. Mitochondria also sequester Ca^{2+}. In addition, proteins that bind and sequester calcium, known as calcium-binding proteins, are present in the cytosol. Excitable cells such as nerve cells and muscle cells have an Na^+/Ca^{2+} exchanger, which becomes active at high concentrations of intracellular calcium, moving Ca^{2+} out of the cell in exchange for Na^+. Energy for this exchange comes from the movement of Na^+ into the cell down its electrochemical gradient.

DAG activates protein kinase C

The other second messenger produced by hydrolysis of PIP_2 by PLC is DAG. DAG retains the two long fatty acyl chains of PIP_2, so is hydrophobic and remains anchored in the lipid bilayer. There it functions as an activator of a family of protein kinases known as the **protein kinase C (PKC)** family (Figure 4.10b (iii)). Members of the PKC family are involved in many signalling processes in cell growth and metabolism and more than one form of PKC may be expressed within the same cell.

In the absence of stimulation, PKCs are present in the cytosol as soluble, inactive proteins. On stimulation of the appropriate GPCR, the increase in cytosolic Ca^{2+} initiated by IP_3 (as described above) causes PKC to translocate to the cell membrane. Once at the membrane, PKC is activated by DAG.

Active PKC can phosphorylate many different intracellular proteins (on specific serine or threonine residues), thereby affecting their activity. Which proteins are targets for PKC depends on the cell type. Target proteins include Ca^{2+}-ATPases and the Na^+/Ca^{2+} exchanger, providing a mechanism for controlling the intracellular concentration of calcium.

4.6.4 Naturally occurring toxins can affect G protein function

Two naturally occurring toxins that exert their effects by interfering with cell signalling by G proteins are cholera toxin and pertussis toxin.

Cholera toxin is produced by the bacterium *Vibrio cholerae* (which causes cholera), and leads to serious malfunction of the cells of the intestine. Cholera is characterised by extreme diarrhoea; a vast volume of water moves from the blood into the gut and is expelled, because of changes in the ion channels in the cell membrane of intestinal epithelial cells. The severe dehydration that results is often fatal.

Biochemical studies have shown that cholera toxin irreversibly activates adenylate cyclase in affected cells, by binding to and modifying the structure of the α subunit of the stimulatory G protein.

■ What is the effect of irreversible activation of the G protein α subunit on cAMP levels in a cell?

□ It causes elevation of cAMP levels, thus effectively keeping the signalling cascade 'turned on' (Figure 4.10b (ii)).

High cAMP levels open channels that allow ions to flood out of gut epithelial cells into the gut lumen. This in turn draws large amounts of water into the gut lumen by osmosis (Section 2.7.1), causing the severe diarrhoea.

The toxin produced by the bacterium *Bordetella pertussis*, which causes whooping cough, also acts on G proteins. Pertussis toxin acts on the α subunit of G proteins that activate phospholipase C, causing them to remain irreversibly bound to GDP.

■ Is a G protein irreversibly bound to GDP permanently turned on, or permanently turned off?

□ It is permanently turned off; the α subunit is inactive when it is bound to GDP.

The inhibition of this signal transduction pathway by pertussis toxin has a number of effects, including disruption of recruitment of immune system cells to infected tissues.

Summary of Section 4.6

- The family of G protein-coupled receptors have a characteristic 7-helix transmembrane structure.
- Targets of activated G proteins include (i) ion channels, (ii) adenylate cyclase, which catalyses the production of cAMP, and (iii) phospholipase C, which hydrolyses PIP_2 to produce two intracellular second messengers, IP_3 and DAG.

- cAMP activates cAMP-dependent protein kinase (PKA), which phosphorylates a number of intracellular proteins including some ion channels, some enzymes involved in metabolism, and CREB, thereby activating or repressing transcription of particular genes. cAMP is broken down by cAMP phosphodiesterases.
- IP_3 is released into the cytosol and stimulates the release of Ca^{2+} from ER stores via activation of the IP_3 receptor on the membrane of the ER. Calcium is a ubiquitous second messenger which binds to proteins such as calmodulin, which itself then binds to and activates a number of Ca^{2+}/calmodulin-dependent protein kinases (CAM kinases). The CAM kinases phosphorylate diverse intracellular proteins.
- Termination of a calcium signal involves dephosphorylation, and hence inactivation, of IP_3 and removal of the calcium from the cytosol by pumping it out of the cell or into intracellular stores.
- DAG activates protein kinase C (PKC), which phosphorylates other intracellular signalling proteins.
- Cholera toxin and pertussis toxin disrupt signal transduction by G proteins.

4.7 Signal transduction by enzyme-linked receptors

In recent years, a myriad of different enzyme-linked receptors have been identified. The ligands that act via these receptors are predominantly cytokines and growth factors (Table 4.1), such as epidermal growth factor (EGF, which stimulates the proliferation of epithelial cells and some other cells). Enzyme-linked receptors were first identified through their role in the control of cell growth, proliferation, differentiation and cell survival; and their variety mirrors that of the cytokines and growth factors that signal through them.

Enzyme-linked receptors form families of related molecules. Structurally, the enzyme-linked receptors characteristically have only one transmembrane domain, a single α-helix that links the extracellular and cytosolic domains. As has already been mentioned, these receptors can be divided into two broad classes: those that have intrinsic enzyme activity (i.e. the cytosolic domain of the receptor itself has catalytic activity), and those that interact with (recruit) a separate cytosolic or cell membrane-bound enzyme. Within these two groups there are further subtypes, but relatively few different types of enzyme activity are associated with these receptors. Some enzyme-linked receptors have, or are linked to, tyrosine phosphatase activity, others to serine/threonine kinase activity; but by far the most abundant are those that function as, or which are linked to, tyrosine kinases.

4.7.1 Receptors with intrinsic enzymatic activity

For receptors with intrinsic enzymatic activity, the basic model of activation is that binding of the signalling molecule to the extracellular domain induces interaction between two or more of the receptor proteins, with the most common scenario being dimerisation (i.e. the formation of a complex of two such receptors, namely a dimer). Often the signalling molecule also forms a dimer, facilitating the dimerisation of the receptor. The two receptors can either be of the same type, in which case they form a *homodimer*, or they can be different subunits of the same receptor or two different receptors, and form a *heterodimer*.

To illustrate how such receptors are activated, consider the **receptor tyrosine kinases (RTKs)**, the major type of receptor with intrinsic enzyme activity, which have, as their name suggests, intrinsic tyrosine kinase activity. The ligands for RTKs include a number of different signalling molecules, mainly growth factors, which typically bind as a dimer. The resulting dimerisation of RTKs leads to close contact between the cytosolic domains of the receptors, and each receptor protein phosphorylates the other (autophosphorylation) at tyrosine residues specific for that receptor (Figure 4.14).

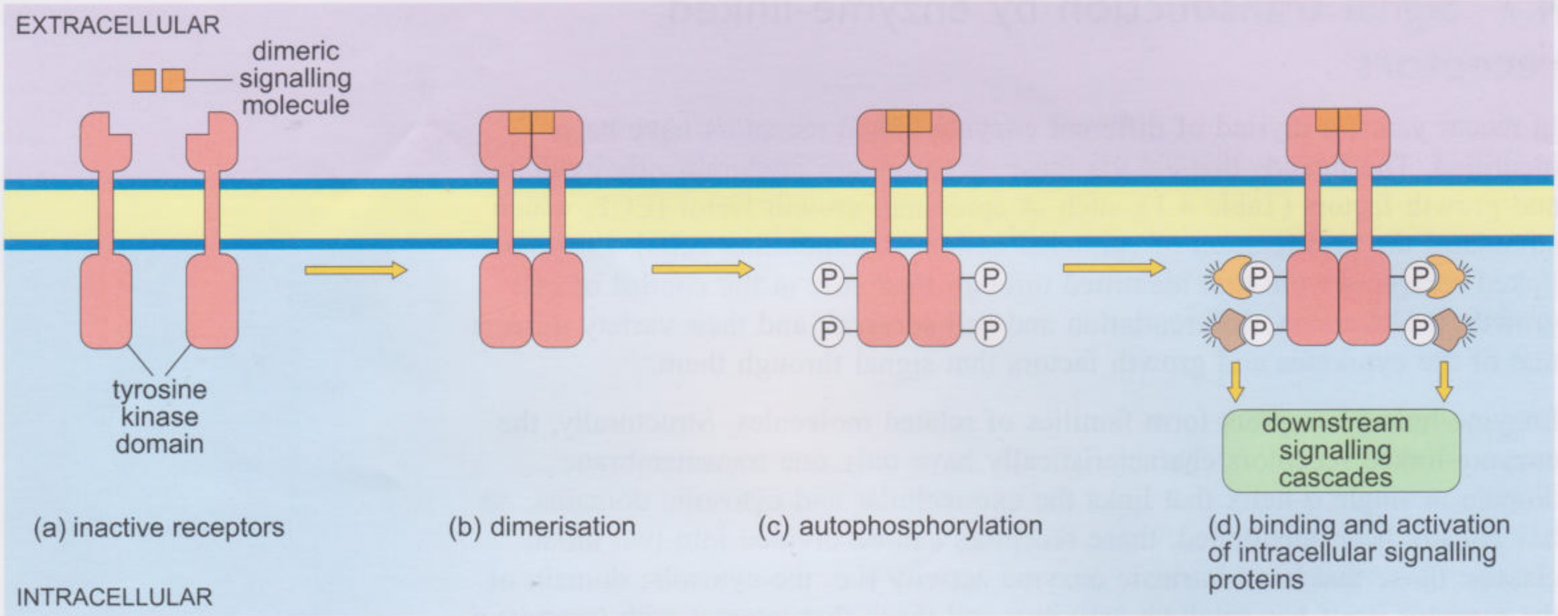

EXTRACELLULAR
dimeric signalling molecule
tyrosine kinase domain
P
downstream signalling cascades
(a) inactive receptors
(b) dimerisation
(c) autophosphorylation
(d) binding and activation of intracellular signalling proteins
INTRACELLULAR

Figure 4.14 Ligand binding to receptors with intrinsic enzyme activity commonly leads to dimerisation, which in turn allows each receptor to phosphorylate specific amino acid residues on the other. The phosphorylated residues form binding sites for other intracellular signalling proteins that are activated on binding and subsequently initiate further downstream signals. The example shown here is a receptor tyrosine kinase (RTK), in which the phosphorylated residues are tyrosines. (a) Inactive receptors prior to binding; (b) binding of signalling molecules to receptors causing dimerisation; (c) autophosphorylation of specific tyrosine residues; (d) binding and activation of other intracellular signalling molecules.

This initial phosphorylation event activates the receptors and can increase intrinsic kinase activity, causing other tyrosine residues to be phosphorylated elsewhere in the cytosolic domain and/or in other proteins. The phosphorylated tyrosine residues (called **phosphotyrosines**) in the activated receptor act as specific binding sites for other intracellular signalling proteins which initiate a diverse and complex set of intracellular cascades leading to complex cell responses such as cell differentiation.

Although there are a large number of proteins that interact with the phosphotyrosines on activated RTKs, they can be divided into two main classes. The first are **adaptor proteins**, which link the receptors to other signalling molecules but have no signalling properties themselves, and the second are proteins that have enzyme activity themselves. The latter group includes two lipid-modifying enzymes: phospholipase C-γ (which is a cytosolic PLC), and an enzyme known as PI3-kinase (phosphatidylinositol 3-kinase). Although these proteins are very different, they each contain a highly conserved polypeptide domain that binds to a distinct sequence of amino acids that surround the phosphotyrosines on the cytosolic domain of the receptor.

Before going on to examine one particular well-studied example of a signal transduction pathway downstream of RTKs, it is appropriate to consider some of the protein–protein interactions upon which such signalling mechanisms depend. The adaptor proteins mentioned above consist of a number of binding domains, through which they interact with other proteins, allowing them to bring those proteins together to form a complex and facilitating their interaction and catalytic function. However, signalling proteins that themselves have enzyme activity have both a catalytic domain and one or more binding domains.

There are, in fact, a small number of types of binding domain that are common to many signalling proteins, which bind to specific targets in other proteins. These include two domains that you have met already in relation to the Src tyrosine kinase, the SH2 and SH3 domains (Section 1.8.5). SH2 (for Src homology 2) domains bind to specific phosphotyrosines in a variety of different proteins, while SH3 (for Src homology 3) domains bind to proline-rich regions in other proteins. Another type of binding domain, called the phosphotyrosine binding (PTB) domain, like SH2, binds phosphotyrosines. The selectivity of recognition of a particular protein by a binding domain depends on the context of the target binding site; that is, an SH2 domain in a particular protein will not bind to just any phosphotyrosine, but will only recognise and bind to those that are within a defined sequence motif. In the case of the SH2 domain of Src, this sequence motif is pYXXI, where pY is phosphorylated tyrosine, X is a hydrophilic amino acid and I is isoleucine.

Signalling from RTKs via Ras protein

Most RTKs, including the growth factor receptors, have signal transduction pathways that include the small intracellular signalling protein known as the **Ras protein** (so named because the *ras* gene was first identified in the **ra**t **s**arcoma virus). These pathways are possibly the most widely studied of all signal transduction pathways because the cellular response is cell proliferation or differentiation, and mutations in proteins involved often underlie the inappropriate growth of cells that occurs in cancer.

The Ras protein is a member of a very large family of proteins which are monomeric GTP-binding proteins (or *small GTPases*), distinct from the trimeric G proteins you learnt about earlier in this chapter (Section 4.6). Ras is tethered at the cytosolic surface of the cell membrane by a lipid anchor, and closely resembles the α subunit of a trimeric G protein. Like the α subunit, Ras cycles between an activated state when GTP is bound and an inactive state when GDP is bound. Ras activity is stimulated by cytosolic proteins known as guanine nucleotide exchange factors (GEFs), which promote the exchange of GDP for GTP, and is terminated by GTPase-activating proteins (GAPs), which increase the GTPase activity of Ras. The cycling between inactive and active Ras is shown in Figure 4.15.

How is the signal transduced from the activated RTK to Ras? Two cytosolic proteins are involved: an adaptor protein known as Grb2 (**gr**owth factor receptor-**b**ound protein **2**, pronounced 'grab2'); and a GEF known as Sos (Figure 4.16). Grb2 consists of little more than two SH3 domains flanking an SH2 domain (Figure 4.17). It binds to the phosphotyrosine sequence motif on the activated receptor via its SH2 domain, and one of its SH3 domains binds to Sos (which contains the appropriate proline-rich sequence motif). Sos then activates Ras by encouraging inactive Ras to exchange its GDP for GTP (Figure 4.16).

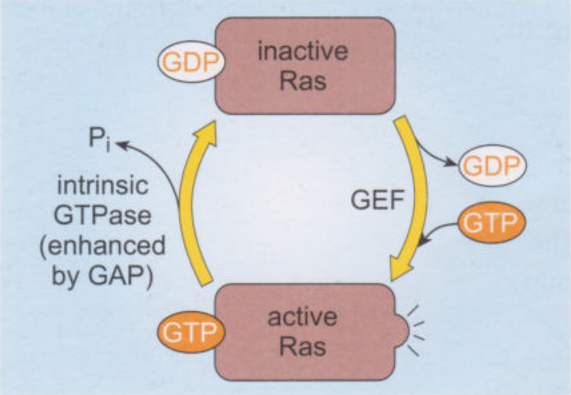


Figure 4.15 Activation and inactivation of Ras. Replacing GDP with GTP, often with the help of guanine nucleotide exchange factors (GEFs), results in activation of Ras. The GTP is hydrolysed back to GDP by intrinsic GTPase activity, enhanced by the action of GTPase activating proteins (GAPs).

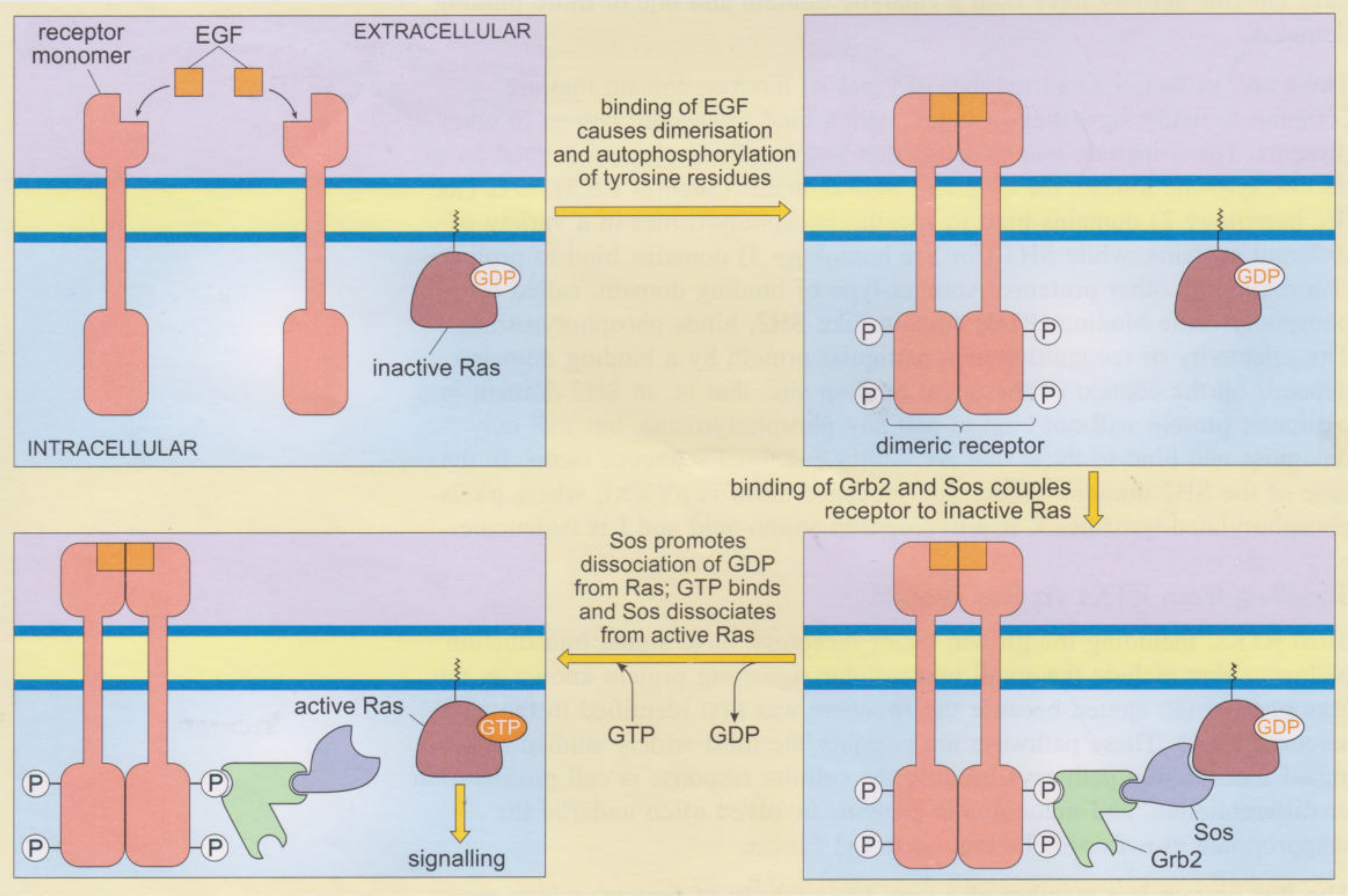

receptor monomer
EGF
EXTRACELLULAR
GDP
inactive Ras
INTRACELLULAR
binding of EGF causes dimerisation and autophosphorylation of tyrosine residues
GDP
P
P
P
P
dimeric receptor
binding of Grb2 and Sos couples receptor to inactive Ras
GDP
P
P
P
P
Sos
Grb2
Sos promotes dissociation of GDP from Ras; GTP binds and Sos dissociates from active Ras
GTP
GDP
active Ras
GTP
P
P
P
P
signalling

Figure 4.16 Activation of tyrosine kinase receptors (in this case, by the growth factor EGF) leading to the binding of Grb2, Sos and activation of Ras protein (steps described in text).

Once Ras is activated, it initiates a cascade of phosphorylation reactions involving a series of kinases known as **mitogen-activated protein kinases (MAP kinases)**. MAP kinases are so-called because they were shown to be activated by mitogens, i.e. intercellular signalling molecules that stimulate cells to divide. Ras activates the first MAP kinase, which phosphorylates the next kinase in the cascade, which in turn phosphorylates, and activates, a third MAP kinase. This activated MAP kinase goes on to phosphorylate other target proteins, including transcription factors (Figure 4.18).

If the activity of Ras is inhibited, for example by injecting anti-Ras antibodies into the cell, the normal response of the cell to an extracellular ligand is blocked. Likewise, if the activity of Ras is permanently switched on, then the cells continue to react as though there was constant activation of the tyrosine kinase receptor. Inappropriate activation of Ras is one of the underlying causes of the uncontrolled growth of some cancerous cells. If there is a mutation in the *ras* gene, such that the Ras protein produced by the cell becomes hyperactive, then the intracellular kinase cascade that is downstream of the hyperactive Ras also remains active even in the absence of an extracellular ligand such as a growth factor. Thus, if the cellular response is growth, the mutant Ras protein induces uncontrollable cell growth. Mutations in *ras* occur in about 30% of all cancers, and many

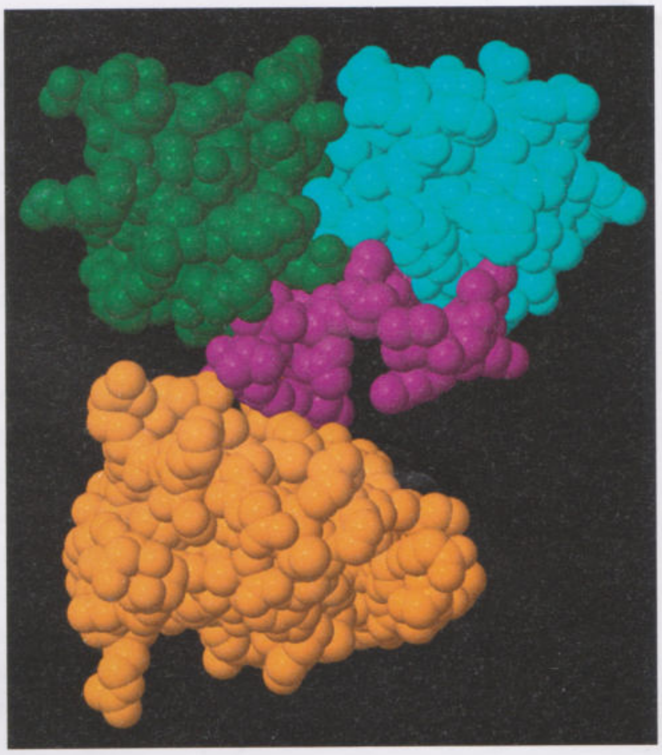


Figure 4.17 Space-filling model of mammalian Grb2 (Protein Data Bank entry 1gri). Two SH3 domains (coloured green and blue) are connected to an SH2 domain (coloured orange).

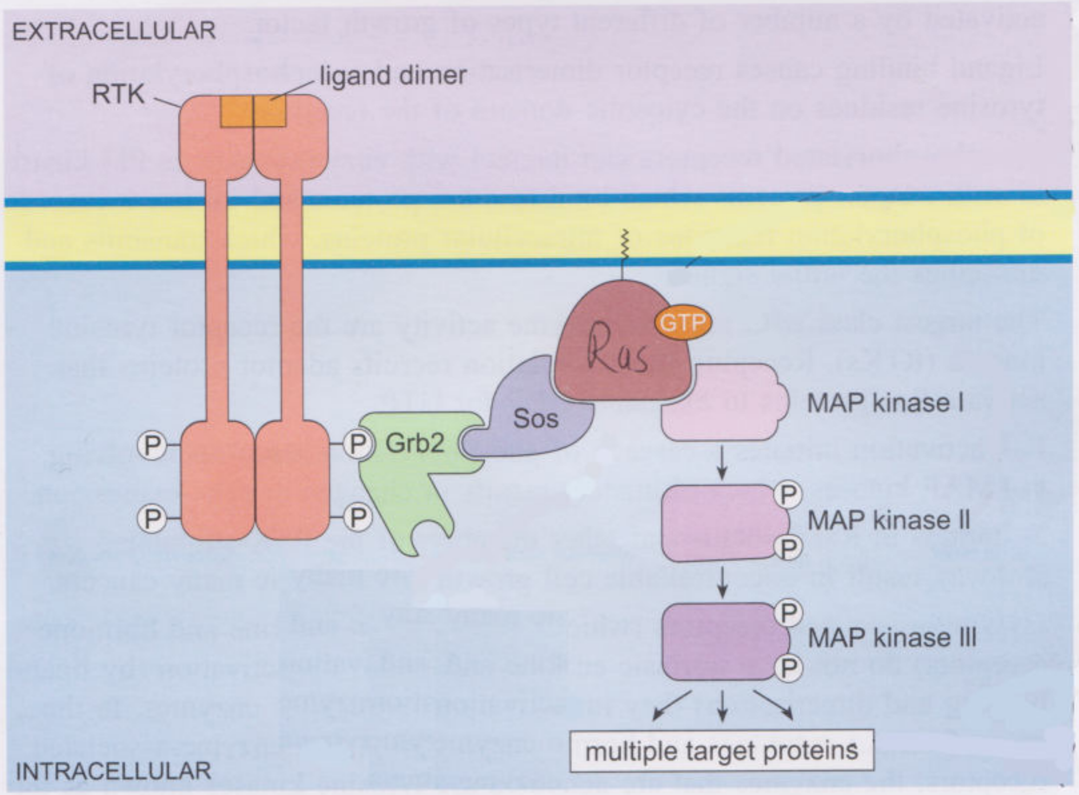


Figure 4.18 Signalling through the Ras–MAP kinase pathway downstream of growth factor binding to an RTK.

other cancers involve mutations in genes encoding other members of the same signalling pathway, such as the MAP kinases downstream of Ras.

4.7.2 Enzyme-associated receptors

In the case of those enzyme-linked receptors that do not have intrinsic enzyme activity, which includes a number of cytokine and hormone receptors, ligand binding causes stimulation of separate tyrosine kinases resident in the cytosol. Once again, ligand binding causes receptor dimerisation.

In the case of certain cytokines and hormones that signal via enzyme-associated receptors, the enzymes that are recruited to the receptor dimer are soluble intracellular tyrosine kinases belonging to a particular class of proteins known as the JAK (janus kinase) family. Once activated, these JAKs then phosphorylate and activate regulatory proteins known as STATS (for **s**ignal **t**ransducers and **a**ctivators of **t**ranscription). Activated STATs migrate to the nucleus, where they stimulate the transcription of specific target genes. Different cytokine and hormone receptors evoke different cell responses by activating different STATs.

Summary of Section 4.7

- Enzyme-linked receptors can be divided into two broad functional classes: those that have intrinsic enzyme activity and those that activate separate cytosolic or cell membrane-bound enzymes.
- Enzyme-linked receptors have a single transmembrane domain and are activated by a number of different types of growth factor.
- Ligand binding causes receptor dimerisation and autophosphorylation of tyrosine residues on the cytosolic domain of the receptor.
- The phosphorylated receptors can interact with enzymes such as PI3-kinase or with adaptor proteins which bind to other proteins and initiate a cascade of phosphorylation reactions of intracellular proteins, which transmits and multiplies the initial signal.
- The largest class with intrinsic enzyme activity are the receptor tyrosine kinases (RTKs). Receptor phosphorylation recruits adaptor proteins that activate Ras proteins to exchange GDP for GTP.
- Ras activation initiates a cascade of phosphorylation reactions involving the MAP kinases, which ultimately results in changes in gene expression.
- Mutations in Ras proteins and other members of the RTK-stimulated pathway result in uncontrollable cell growth and underlie many cancers.
- Enzyme-associated receptors (which include some cytokine and hormone receptors) do not have intrinsic enzymatic activity; on activation (by ligand binding and dimerisation) they in turn activate cytosolic enzymes. In the case of certain cytokines and hormones that signal via enzyme-associated receptors, the enzymes that are activated are tyrosine kinases known as the JAKs, which phosphorylate and activate regulatory proteins known as STATS, which in turn affect transcription of specific target genes.

4.8 Interaction and integration in signal transduction pathways

This chapter has described just a few of the major intracellular pathways that transduce a signal from receptor activation to the cellular response. These pathways have been described, however, in a rather simplistic manner, as if they operate in isolation. In reality, there are many points at which different signalling pathways interact. To help you appreciate some of the complexity of interactions between signalling pathways, Figure 4.19 illustrates some of the interactions between pathways downstream of GPCR and RTK activation. Be assured that you are not required to learn or recall the details in this figure.

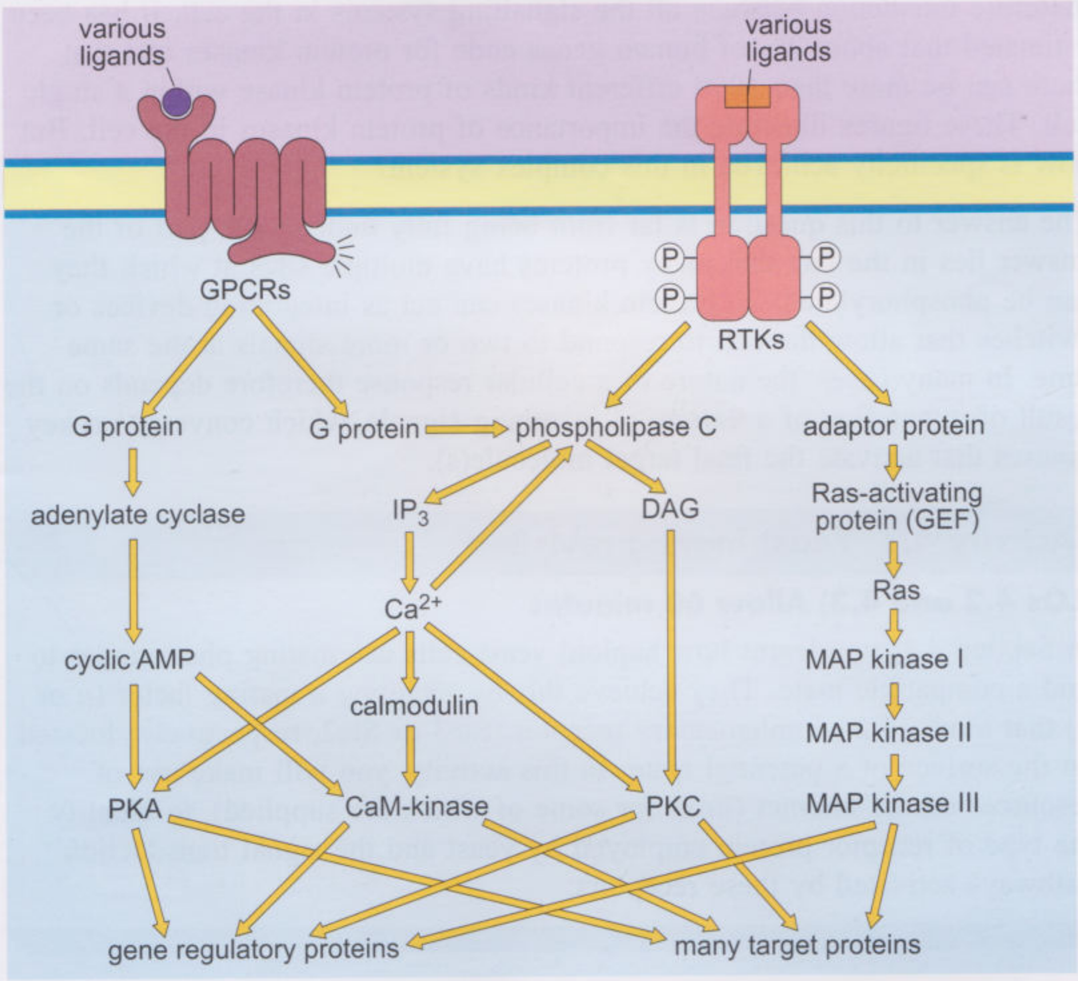


Figure 4.19 Interactions between the major signalling pathways from the GPCRs and RTKs.

This diagram summarises the signalling pathways from both GPCRs and RTKs and their interactions. Various ligands bind to GPCRs, resulting in activation of G proteins. In turn, G proteins activate adenylate cyclase, which produces cyclic AMP. cAMP activates PKA which has downstream effects on gene regulatory proteins and many other target proteins; cAMP also activates CAM kinases, which likewise act on both gene regulatory proteins and many other target proteins. In addition to their role in activating adenylate cyclase, G proteins activate phospholipase C, which produces the two second messengers IP_3 and DAG. IP_3 acts to regulate calcium levels, which influence the kinases PKA and PKC and also calmodulin, which in turn regulates the activity of CAM kinases. The other product of PLC activity, DAG, also affects PKC activity. In addition to PLC activation via G proteins, various ligands bind to RTKs, leading to activation of PLC, with the various downstream effects just mentioned. RTK activation also leads to activation of Ras, and signalling via the Ras–MAP kinase pathway affects gene regulatory proteins and many other target proteins.

Although there are a number of places where the different pathways can interact, most of this interaction is mediated by the protein kinase components of these pathways, which can phosphorylate, and thereby regulate, the components of other signalling pathways as well as their own. There is therefore interaction between all the signalling systems in the cell. It has been estimated that about 2% of human genes code for protein kinases and that there can be more than 1000 different kinds of protein kinase within a single cell. These figures illustrate the importance of protein kinases in the cell. But how is specificity achieved in this complex system?

The answer to this question is far from being fully understood; part of the answer lies in the fact that many proteins have multiple sites at which they can be phosphorylated. So protein kinases can act as integrating devices or switches that allow the cell to respond to two or more signals at the same time. In many cases, the nature of a cellular response therefore depends on the result of integration of a variety of incoming signals, which converge on key kinases that activate the final target molecule(s).

Activity 4.1 Yeast mating revisited

(LOs 4.2 and 4.3) Allow 60 minutes

In Section 4.1, you learnt how haploid yeast cells use mating pheromones to find a compatible mate. They achieve this by secreting a mating factor (*a* or *α*) that binds to a complementary receptor (Ste3 or Ste2, respectively) located on the surface of a potential mate. In this activity, you will make use of resources on the internet (links for some of which are supplied), to identify the type of receptor protein employed by yeast and the signal transduction pathways activated by these receptors.

4.9 Final word

Now that you have reached the end of this chapter, you will have an appreciation of the complexities of cell signalling and the diverse signal transduction pathways involved. Cells need to communicate and respond appropriately to survive and thrive. The arrival of a signalling molecule is only the first step in a cascade of cellular events that can bring about widespread or discrete changes in cell function and behaviour. Furthermore, the interaction of many signal transduction pathways allows a cell to fine-tune its response. You have also learnt that a given signalling molecule will not necessarily produce the same outcome in every cell. The way a cell responds to a signal is a function of the types of receptors expressed by it and the transduction pathways coupled to them.

4.10 Learning outcomes

4.1 Describe the variety and properties of signalling molecules and the routes by which they reach their receptors/target proteins.

4.2 Describe the mechanisms used by activated receptors to initiate signal transduction.

4.3 Describe, with examples, how signalling pathways amplify, integrate and regulate signalling cascades.

Chapter 5 Cell movement

5.1 Introduction

Cells are not static; an enormous range of internal cellular movement and related changes in cell shape take place nearly all the time in nearly all cell types. Changes in cell shape are often related to function: for example, the processes of phagocytosis, endocytosis and exocytosis (described in Section 2.9) entail considerable distortion of the cell shape. Some cells have moving parts such as flagella, which can propel the cell through the extracellular medium; others can migrate from one place to another through remodelling of their cytoskeleton and interactions with the extracellular environment. This chapter is concerned with the cellular mechanisms that make these movements possible, and begins by considering the different kinds of cellular movements and why they are necessary or advantageous.

5.1.1 The variety of cellular movements

Intracellular movement is required to move proteins, metabolites and, in eukaryotes, organelles, to where they are needed inside the cell. While most prokaryotes are small enough that molecules can generally reach their internal targets by diffusion, eukaryotic cells are large enough that diffusion is not sufficiently rapid, and need an internal transport system. When a living eukaryotic cell is observed under the microscope, its cytoplasm is seen to be constantly moving or 'streaming', a phenomenon called **cyclosis** (also referred to as cytoplasmic streaming), which ensures circulation of nutrients, proteins and other cellular materials. Cyclosis is particularly evident in large plant cells.

When living eukaryotic cells are examined microscopically, their organelles, such as the mitochondria, can be seen to make small jerky, jumping movements, called *saltatory* movement, as they are manoeuvred along cytoskeletal filaments. Vesicles containing proteins, packaged in the Golgi for export, are also transported along cytoskeletal filaments to the cell surface for secretion, while material that has entered the cell via endocytosis is shuttled towards a site of utilisation within the cell. As well as these 'housekeeping' processes, intracellular movements and changes in cell shape are vital during cell division (mitosis), when duplicate chromosomes are separated and pulled to opposite poles of the cell prior to separation of the daughter cells (Book 1, Section 4.3 and Book 3, Section 1.3).

In multicellular organisms, microvilli (Figure 5.1a), which are finger-like projections found in very large numbers on some kinds of epithelial cell, move passively in response to tissue movements or movement of extracellular materials; in the gut, they serve to increase the surface area of the epithelial cells and facilitate absorption of nutrients. While microvilli only move passively, the movement of cilia – which are larger projections and have a very different construction (Figure 5.1b) – is controlled by the cell. Cilia are typically used to propel fluids or substances over the surface of the cell, as in epithelial cells lining the bronchial tubes of the respiratory system, or to generate movement of the cell, as in the ciliated *Paramecium* shown in

Figure 5.1c. The structure and function of cilia are described in detail in Section 5.4.4.

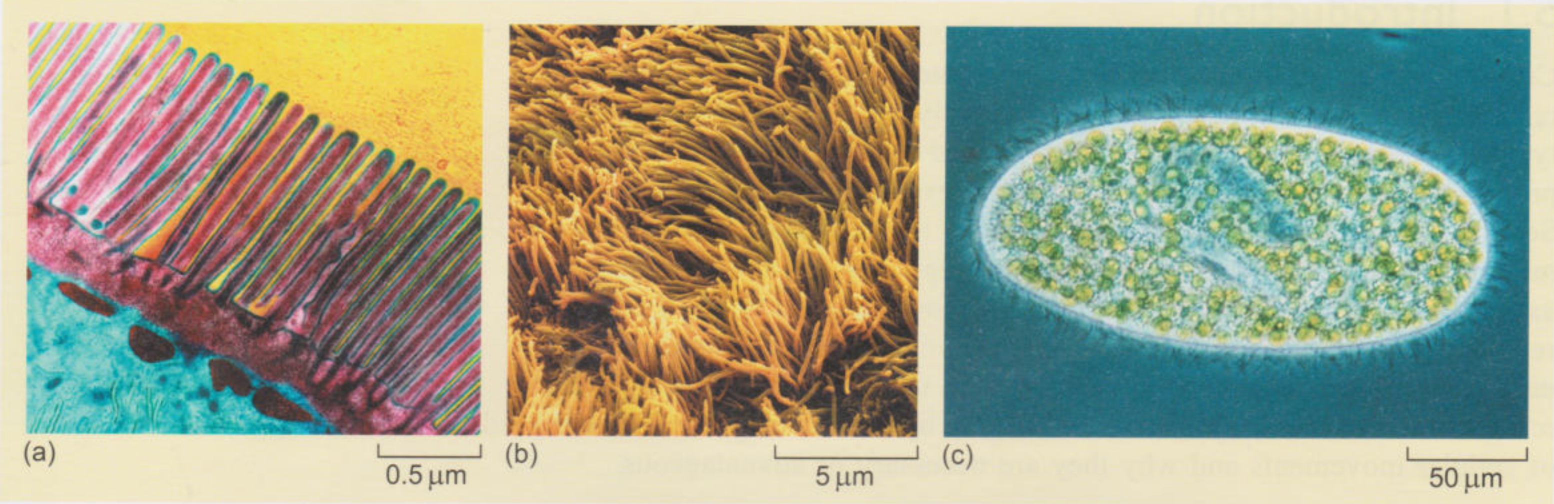


Figure 5.1 (a) False colour transmission electron micrograph (TEM) of microvilli on the epithelial cells lining the intestine; microvilli are typically 0.1 µm in diameter and 1 µm long. (b) Scanning EM of a field of cilia in the bronchial lining. (c) Light micrograph of the unicellular, ciliated protist, *Paramecium* sp.

A different kind of movement is evident in animal muscle – specialised tissue composed of cells that are capable of coordinated contraction and relaxation. Muscle contraction (discussed in Section 5.3) is essential to many physiological processes, including locomotion, circulation of blood and other fluids and movement of material through the intestine during digestion.

However, perhaps the most obvious form of cellular movement is the ability of certain cells to migrate from one place to another. Motility allows many unicellular and simple multicellular organisms to respond to their environment and is generally important for feeding or escape from a threat, or for some other survival strategy.

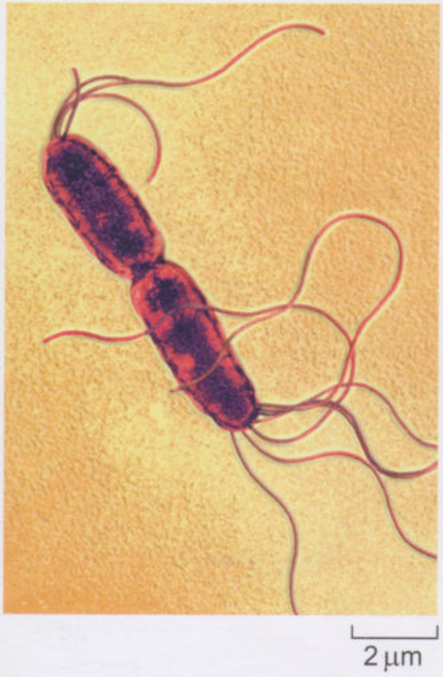


Figure 5.2 False colour scanning EM of a bacterium (*Salmonella typhimurium*) with flagella.

Among the eukaryotes, protists adopt a variety of strategies to move within their environment. Some unicellular protists can 'swim'; that is, they can move through a fluid environment, and do not require a solid support or substrate. These swimming movements are generated by movement of cell surface projections such as flagella and cilia. Among prokaryotes there are also many motile species. Though superficially their movement appears to be generated by similar means (i.e. flagella), as you will see later, the structures and mechanisms involved are in fact very different. Figure 5.2 shows a bacterium that has a number of flagella, which are required for its movement.

■ What conditions might attract a single-celled protist or bacterium and what conditions might repel these cells?

□ Generally, they move towards favourable environmental conditions, such as high concentrations of nutrients, and away from unfavourable conditions, such as extremes of pH (acidity or alkalinity).

Whilst cell motility is rare in higher plants, in complex multicellular animals cell motility enables particular cells to assemble where they are needed or to make contact with other cells; for example during an immune response, during fertilisation or during development when important intercellular contacts are established. Cells within complex animals do not usually have the capacity to 'swim' in the way that certain unicellular protists and prokaryotes can; though there are exceptions.

■ Unlike most cells in higher animals, sperm have the ability to swim. Why is this necessary?

□ Sperm cells use their single flagellum to swim long distances to reach an ovum (egg).

The movement of individual cells in multicellular organisms usually depends on specific interactions with a solid substrate and is more of a creeping or crawling motion, sometimes described as 'amoeboid'. As the name implies, this kind of movement is also demonstrated by the protist, *Amoeba* (Figure 2.12 in Book 1).

During development, animal cells produced by repeated cell divisions differentiate in a coordinated fashion to produce specialised cell types that form particular tissues adapted for specific functions: for example, neurons in the nervous system; the different types of muscle cell in skeletal muscle, heart muscle and smooth muscle; the different specialised cells of the kidney. By recognising cues (i.e. signals) from surrounding cells and the extracellular matrix, precursor cells are triggered to migrate to the appropriate location in the developing organism where they differentiate and establish appropriate connections with other cells (discussed further in Book 3, Section 1.4).

Many animal cells are largely immotile *in situ* (in position within the organism); however, if they are removed from the organism and cultured under suitable conditions (Book 1, Section 2.2.4), they can creep across the surface of the culture vessel. Thus the capacity for cellular locomotion persists in these cells and can be reactivated.

Despite some superficial similarities, the structures and mechanisms involved in cell motility in eukaryotic and prokaryotic cells are generally quite distinct (Section 5.4). As you will see, in eukaryotic cells, the cytoskeleton and associated proteins are particularly important for both migration (Sections 5.4.3 and 5.4.4) and internal movements (discussed in the following section).

Summary of Section 5.1

- Cellular movements are varied and include internal movement of cellular contents, rearrangements and/or changes in cell shape and translocation or migration of the cell.
- Movements are often an important aspect of cellular function, as in phagocytic cells, bronchial epithelium and sperm; or cell survival, as in many motile unicellular organisms.
- Migration of cells can be achieved by (i) swimming movements, where projections (flagella or cilia) generate the force necessary to propel the cell

through a fluid environment; or (ii) creeping/crawling movements, where contact with a solid substrate is required.

5.2 The cytoskeleton and mechanisms of intracellular movement in eukaryotes

The components of the eukaryotic cytoskeleton were described in Book 1, Section 3.4.2.

- ■ What are the three types of cytoskeletal filament and the proteins of which they are composed?
- □ Microtubules are formed from tubulin, microfilaments are formed from actin and intermediate filaments are formed from a number of different types of intermediate filament proteins.

The proteins that are assembled to form intermediate filaments vary from cell to cell: for example, *keratins* in epithelial cells and *vimentin* in connective tissue. This variation is often exploited in the laboratory to identify different cell types using antibodies specific to particular intermediate filament proteins (Book 1, Box 2.4).

The cytoskeleton is a highly dynamic structure. In the case of animal cells, which lack a cell wall, the scaffolding of microtubules, intermediate filaments and actin filaments (microfilaments) confers cell shape and helps the cell to resist mechanical stresses. The cytoskeleton is also important for changes at the cell surface, such as during phagocytosis, and movement of organelles and other particles within the cell. Actin filaments form the core of microvilli and are also critical in amoeboid movements, while microtubules are a major component of eukaryotic flagella and cilia, involved in swimming movements in motile cells.

5.2.1 Assembly and disassembly of microtubules and microfilaments

Microtubules and microfilaments (actin filaments) are assembled from, respectively, soluble tubulin and actin proteins in the cytosol and are disassembled when they are no longer needed (Book 1, Section 3.4.2). Importantly, microtubule and microfilament assembly are *active* processes; that is, they require energy. Microtubule assembly requires GTP, while formation of actin filaments from actin subunits requires ATP as an energy source.

The term 'polymerisation' is sometimes used to refer to the assembly of cytoskeletal filaments but is avoided here to prevent confusion with the process of covalent linkage of monomeric units to form polymers (Book 1, Section 1.1.1).

A further point to note, of significance with respect to cell movement, is that both microtubules and actin filaments have polarity; that is, the two ends of the filaments are not identical. This polarity arises from the orientation of the subunits in assembly of the filaments, and by convention one end is described as the 'plus' end, and one as the 'minus' end. The plus end of a microtubule or microfilament is so called because subunits are added more rapidly to this end than to the opposite (minus) end. (Note that the 'plus/minus' nomenclature does not refer to positive and negative charges.)

Microfilaments have a diameter of about 6 nm and are typically found in bundles. Most of the soluble actin protein from which microfilaments are assembled is bound by another cytosolic protein called *profilin*. This small protein binds reversibly to actin molecules, forming a profilin–actin complex, which serves as 'reservoir' of actin subunits. When actin filaments are required, profilin dissociates from actin, enhancing filament assembly. Actin assembly can be extremely rapid. For example, during fertilisation of a sea urchin egg, the sperm pierces the coating of the egg by rapidly extending a long thin process called the acrosomal process, formation of which requires 'explosive' assembly of actin filaments at a rate of 10 μm per second.

Microtubules are hollow tubes (about 25 nm outer diameter) formed from parallel tubulin filaments. The basic unit of a microtubule is a dimer of the protein tubulin, composed of one α-tubulin molecule and one β-tubulin molecule (Figure 5.3a), bound to each other very tightly such that they do not readily dissociate. The α- and β-tubulin subunits can each bind a molecule of GTP. When the dimers are in the GTP-bound form, their assembly into microtubules is favoured. Figure 5.3b illustrates how the tubulin heterodimers assemble to form microtubules. Notice that within the microtubule, all the tubulin dimers are orientated in the same direction. Thus the two ends of the microtubule differ chemically and structurally, accounting for microtubule polarity. The end of the microtubule with exposed α subunits is the minus (−) end, while the other end with the β subunits exposed is the plus (+) end.

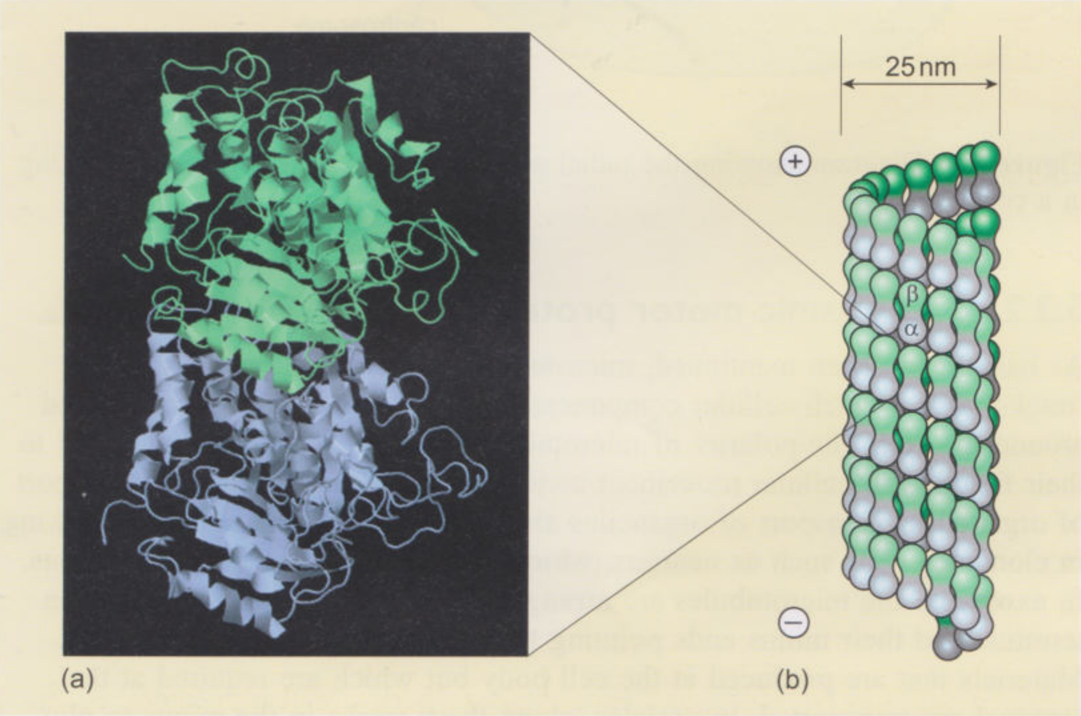


Figure 5.3 Microtubule structure and assembly. (a) A dimer of α-tubulin and β-tubulin (Protein Data Bank file 1tub). (b) Microtubule structure. All the dimers are orientated in the same direction; thus the plus (+) and minus (−) ends of the microtubule are distinct (i.e. the microtubule has polarity).

Microtubule formation originates at a **microtubule organising centre (MTOC)**. Animal cells typically contain a single MTOC called the centrosome, which is usually located near the nucleus, as illustrated in

Figure 5.4. The centrosome consists of a structure called the centriole, two short cylinders of microtubules arranged in a precise configuration, embedded in a matrix of protein. The matrix also includes many ring-shaped structures, made of a different kind of tubulin molecule (γ-tubulin). These γ-tubulin structures form a core from which growing microtubules extend. Microtubules remain stable only if their ends are 'capped' to prevent assembly and disassembly. The minus end of each microtubule is capped by the MTOC, but the plus end is constantly shrinking and growing, unless attachment to capping proteins or cellular structures such as organelles protects and stabilises the end of the microtubule.

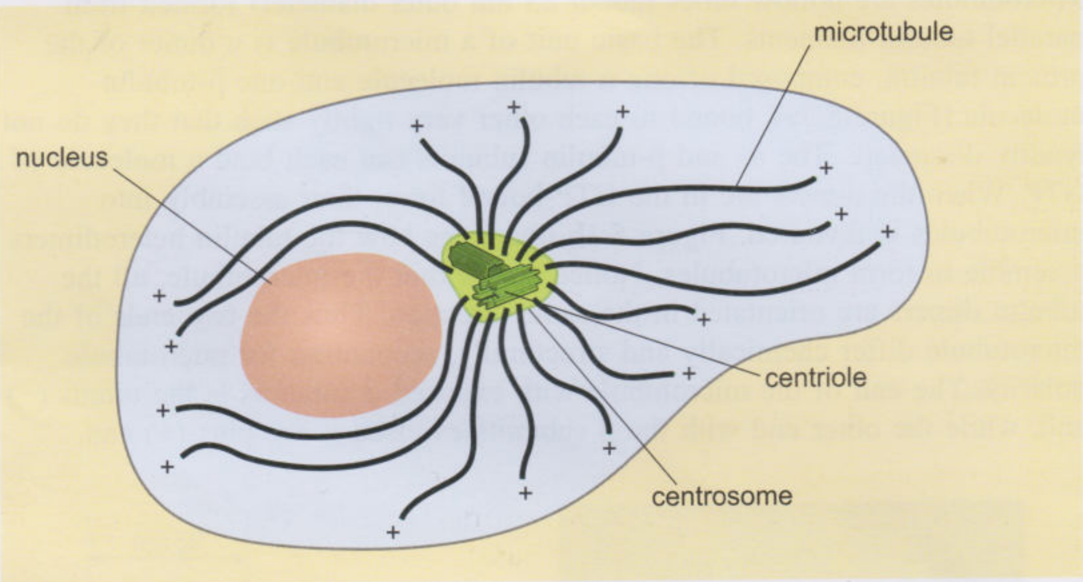


Figure 5.4 Diagram showing the radial arrangement of microtubules originating in a centrosome.

5.2.2 Cytoplasmic motor proteins

As has already been mentioned, microtubules and actin filaments serve as 'tracks' along which cellular components such as organelles are transported around the cell. The polarity of microtubules and microfilaments is crucial to their function in cellular movement as it determines the direction of transport of organelles. Transport of organelles along microtubules is especially striking in elongated cells such as neurons, which have long extensions called axons. In axons, all the microtubules are arranged with their plus ends at the axon terminal and their minus ends pointing towards the cell body (Figure 5.5). Materials that are produced at the cell body but which are required at the terminal are transported, in vesicles, along these tracks in the minus to plus direction. Other materials can be transported in the opposite direction.

How does this transport process discriminate between the two directions? The answer lies in another set of proteins called motor proteins. **Motor proteins** use energy derived from ATP hydrolysis to travel along a microtubule or microfilament, taking their particular 'cargo' with them. Directed intracellular movements, such as cyclosis in eukaryotic cells, or the transport of organelles and vesicles along a nerve axon (Figure 5.5) are all due to motor proteins

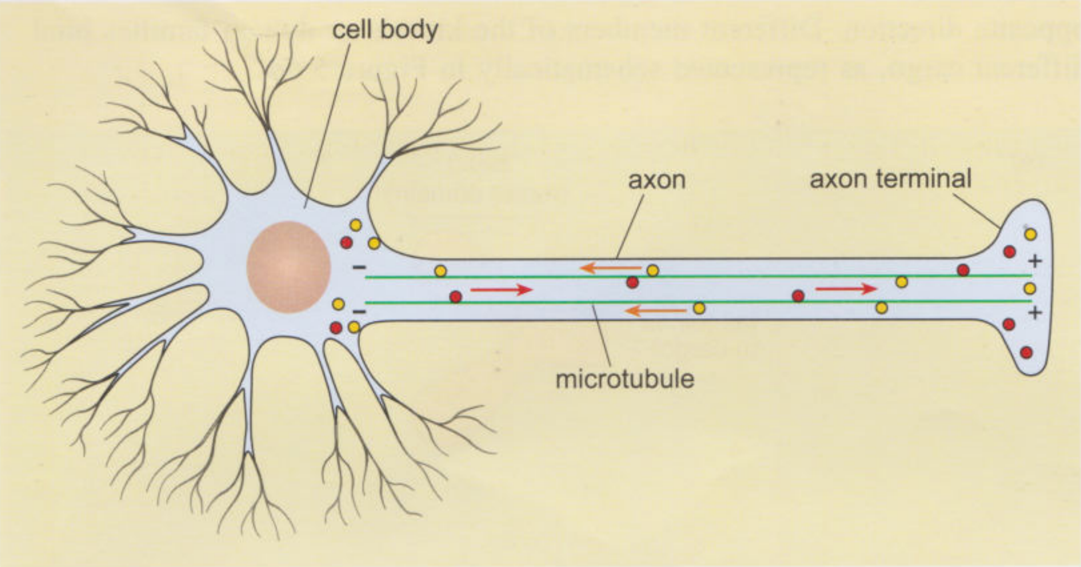


Figure 5.5 Intracellular movements in nerve cell axons. Microtubules in axons are orientated such that their minus ends are closest to the cell body and plus ends extend towards the axon terminal. They function as 'tracks', along which vesicles and organelles are shuttled according to where they are needed.

moving along microtubules or microfilaments. The key point to note is that different types of motor protein operate in each direction.

Most motor proteins, though not all, have a 'head on a stalk' configuration (as depicted in Figure 5.6a) and are often dimers. The head of each subunit in the dimer contains a motor domain, which has ATPase activity and binds to the appropriate track (actin filaments or microtubules). The stalk or tail binds specifically to the appropriate cargo, to other motor proteins or to other 'adaptor' proteins. The specificity of the interaction between the head and the track is such that the motor protein 'faces' the appropriate direction of travel. In some cases, the tail has a regulatory role, folding up to bind reversibly to the head structure, thereby turning it on or off.

Two different families of motor proteins, called **kinesins** and **dyneins**, are able to travel along microtubules (Figure 5.6b), while members of the **myosin** protein family move along actin filaments. Examples of motor proteins and the transport/movement processes in which they are involved are listed in Table 5.1. Note that cells specialised for movement often contain specialised motor proteins: for example, muscle cells contain a specific form of myosin (myosin II), which is involved in contraction and which differs from myosins found in other cell types.

Though members of the kinesin and dynein families of motor proteins all move along microtubules (Table 5.1), they do so in opposite directions. Thus kinesins move towards the plus end of the microtubule and dyneins move towards the minus end (Figure 5.6b). As has already been described, microtubules are typically arranged such that their plus ends are towards the outside of the cell and their minus ends are towards the centre of the cell. Thus, generally speaking, kinesins transport cellular components towards the cell surface, away from the centrosome, and dyneins transport them in the opposite direction. Different members of the kinesin or dynein families bind different cargo, as represented schematically in Figure 5.6b.

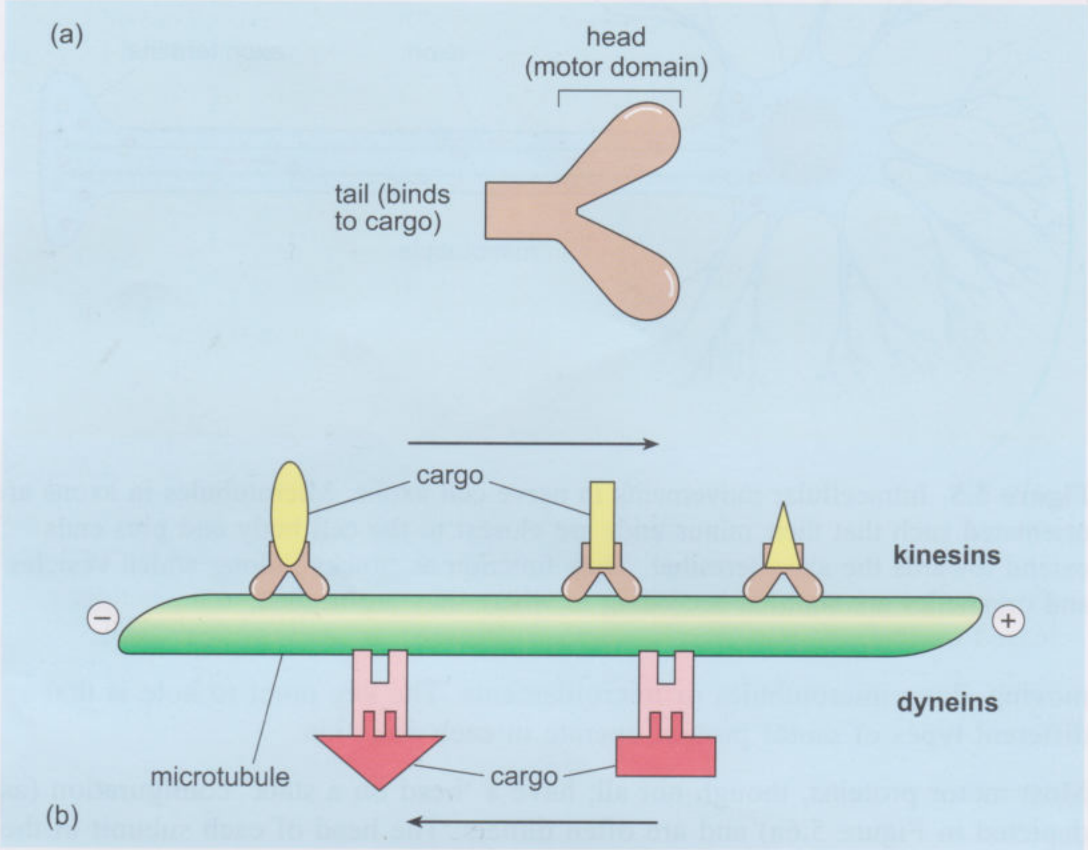


Figure 5.6 (a) A dimeric motor protein with a 'head on a stalk' configuration. The heads consist of a motor domain that binds to the track (microtubules in this case) and have ATPase activity. (b) Schematic of transport by motor proteins along microtubules. Different motor proteins transport cargoes or cell components in different directions.

Table 5.1 Motor proteins involved in cellular movements. Motor proteins exist in many different forms. Myosin I and II, kinesin and dynein actually are general names for different families of motor protein, and other myosin families do exist. Those indicated here have been identified chiefly in animal cells. Different types of myosin are generally present in all animal, plant and fungal cells. Dyneins and kinesins are poorly documented in plants.

Motor protein/family	Transport/movement process	Moves along:
myosin I	vesicle and organelle transport	actin filaments
myosin II	muscle contraction	actin filaments
kinesins	cytokinesis (spindle assembly, movement of chromosomes); vesicle and organelle transport (towards the cell surface)	microtubules
cytoplasmic dyneins	vesicle trafficking; localisation of the Golgi apparatus	microtubules
axonemal dyneins	beating of eukaryotic flagella and cilia	microtubules

How do motor proteins move along microtubules and microfilaments? At its simplest, the function of a motor protein is to move other molecules or to move itself in relation to other molecules. This movement is a form of work and therefore it requires energy, which is provided by the hydrolysis of ATP. An ATP molecule binds to the motor protein and is hydrolysed to ADP, with an accompanying change in the conformation of the protein. This change in conformation affects a part of the protein that is required for binding to the microtubule or microfilament. ATP is therefore acting as both a source of energy and an allosteric effector in this mechanism.

■ What is an allosteric effector?

□ It is a ligand that binds to a specific site on a protein and thereby induces a conformational change in the protein at a different site (Section 1.8.5).

A generalised cycle of motor protein–ATP binding and conformational change is illustrated in Figure 5.7a. In step 1, the binding of ATP to the motor protein results in a conformational change in the protein. In step 2, ATP is hydrolysed to ADP and inorganic phosphate (P_i) is released. The motor protein, now with ADP bound to it, adopts a different conformation again. The free energy released on hydrolysis of the ATP makes this step essentially irreversible, as the energy barrier presented by the reverse reaction is very large. The ADP dissociates from the motor protein (step 3), with the result that the motor protein reverts to its original conformation and is free to bind a further ATP molecule and repeat the whole sequence of events. Because these events are essentially irreversible, the movement of the motor protein continues in the same direction. Note that both ATP and ADP function as allosteric effectors and hydrolysis of ATP is catalysed by the motor.

The key to making motor proteins do work is to couple this cycle of ATP hydrolysis and conformational change to their interaction with the 'track'. Figure 5.7b illustrates how this happens in the case of a kinesin, which has two motor domains that are able to 'walk' along a microtubule by alternately attaching and detaching. The cycle of ATP binding and hydrolysis causes each of the motor domains to cycle between weak and strong binding states. When the active site is empty, binding to the track (in this case, a microtubule) is strong. ATP binding and subsequent hydrolysis causes the motor to detach from the track and frees it to reattach to another site. The conformational change that accompanies ATP hydrolysis and release of ADP helps the motor to bind at a site further along the track in the direction of progress.

The mechanisms involved in intracellular movements of the sort described here have much in common with the mechanisms that bring about the muscular movements that occur in vertebrates. The interaction of myosin with actin filaments in muscle cells is a particularly well-characterised example of motor protein function and is discussed in some detail in relation to muscle contraction in the following section.

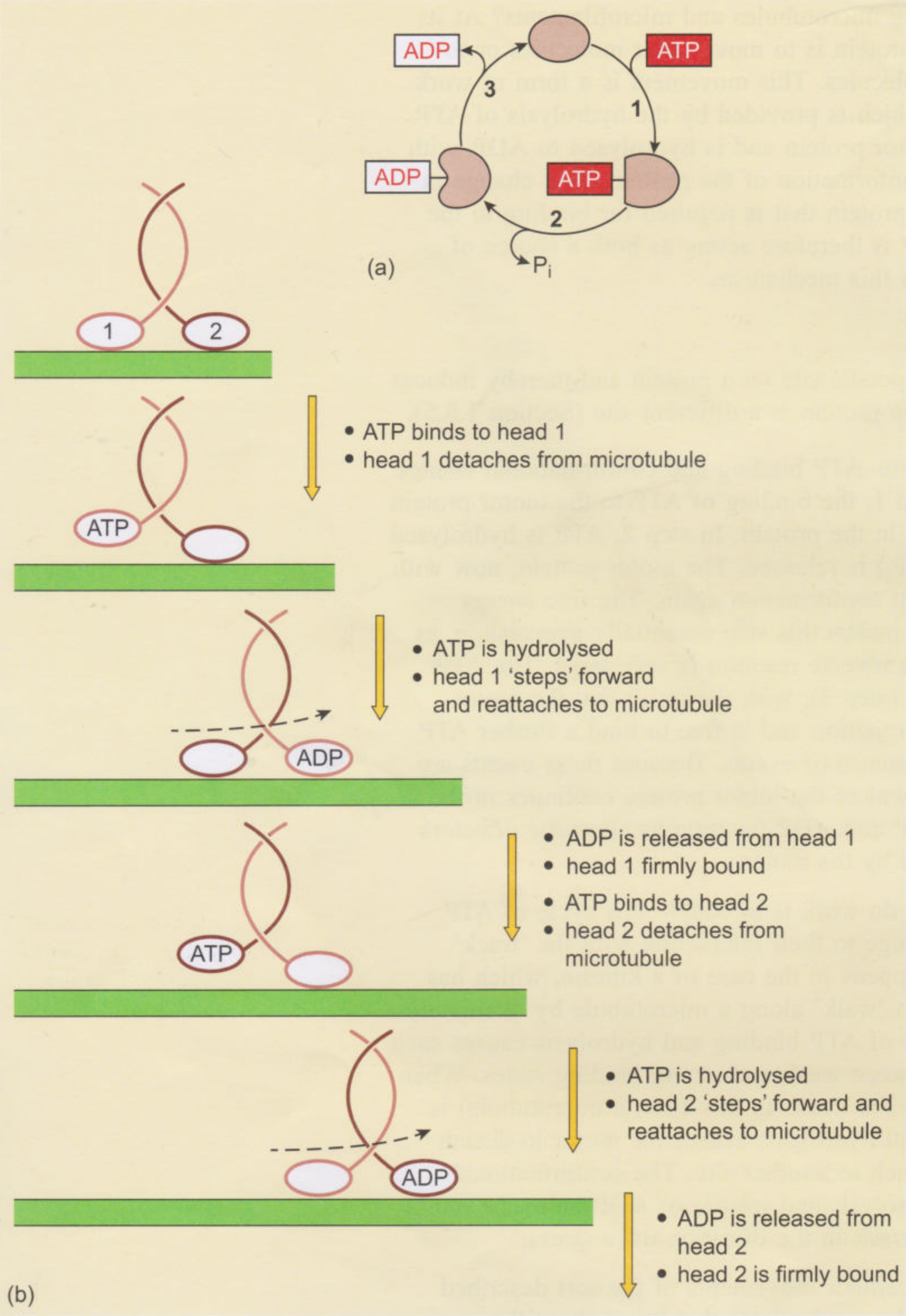

ADP
ATP
3
1
ADP
ATP
2
P_i
(a)
1
2
• ATP binds to head 1
• head 1 detaches from microtubule
ATP
• ATP is hydrolysed
• head 1 'steps' forward and reattaches to microtubule
ADP
• ADP is released from head 1
• head 1 firmly bound
• ATP binds to head 2
• head 2 detaches from microtubule
ATP
• ATP is hydrolysed
• head 2 'steps' forward and reattaches to microtubule
ADP
• ADP is released from head 2
• head 2 is firmly bound
(b)

Figure 5.7 (a) Schematic illustration of the generalised motor protein mechanism showing the cycle of ATP binding and conformational change. The motor protein has ATPase activity and undergoes conformational changes (1) on binding ATP; (2) on hydrolysis of ATP; and (3) on release of ADP. By harnessing the forces generated by these conformational changes, molecular movements can be achieved. (b) A cytoplasmic motor such as kinesin, for example, can use this mechanism to move in one direction along a microtubule. The two heads (motor domains) of kinesin undergo cycles of ATP binding, hydrolysis and release of ADP, as shown for the generalised motor protein mechanism in (a). The conformational changes in the motor protein heads that are associated with this cycle affect the interaction of the kinesin with the microtubule 'track', such that the kinesin 'walks' along the track as represented here.

Summary of Section 5.2

- The cytoskeleton is essential to all eukaryotic cell movements.
- Movement of organelles and cellular components inside a cell is facilitated by motor proteins which recognise specific 'cargo' and carry it along microtubules and actin microfilaments, which serve as tracks. Microtubules and actin filaments have polarity and a particular motor protein only moves along the track in one direction.
- Three major families of motor protein are myosins, kinesins and dyneins. Myosin interacts with actin filaments and is involved in contraction of muscle cells. Kinesins and dyneins interact with microtubules and distribute vesicles and cell organelles inside the cell. Kinesins move towards the 'plus' end and dyneins move towards the 'minus' end of microtubules.
- Motor proteins have a generalised 'head on a stalk' configuration. The head binds to the microtubule or actin microfilament and can bind and hydrolyse ATP. The stalk or tail binds to the cargo. ATP hydrolysis results in a series of conformational changes in the motor protein and hence changes in its binding affinity for the track and, by detaching and reattaching at a different site, the motor protein 'walks' along the track.

5.3 Muscle contraction

There are three different types of muscle, with different functions and subject to different nervous system control. Skeletal muscles support and manipulate the skeleton in locomotion in vertebrates (walking, running, swimming and flying) and are subject to voluntary nervous system control. Smooth muscle has a role in peristaltic movements, e.g. in the digestive system; and cardiac muscle produces contraction of the heart to pump blood around the body. These functions are, of necessity, continuous and are subject to involuntary control by the nervous system.

Peristalsis is a progressive wave of contraction and relaxation of muscles of the gut wall, which moves food along the intestine.

Skeletal, smooth and cardiac muscle are very different in appearance and structure but they share a common mechanism of contraction, which involves actin and myosin filaments. The actin filaments are similar to those of the cytoskeleton, while the muscle myosin filaments are composed of myosin II motor protein (see Table 5.1). In muscle, myosin functions not only as a motor protein but, by virtue of its assembly into filaments, it also has a structural function. A myosin II molecule resembles a golf club in appearance. It has a globular head which has ATPase activity, attached via a flexible 'hinge' region to a long 'tail'. Muscle myosin forms a dimer of a pair of identical myosin molecules held together by their tails with the heads bent to the side (Figure 5.8a). Clusters of these myosin dimers bind to each other through their coiled tails, forming a bipolar myosin filament like a double-headed arrow, with all the globular heads sticking out at the surface in a spiral pattern (Figure 5.8b).

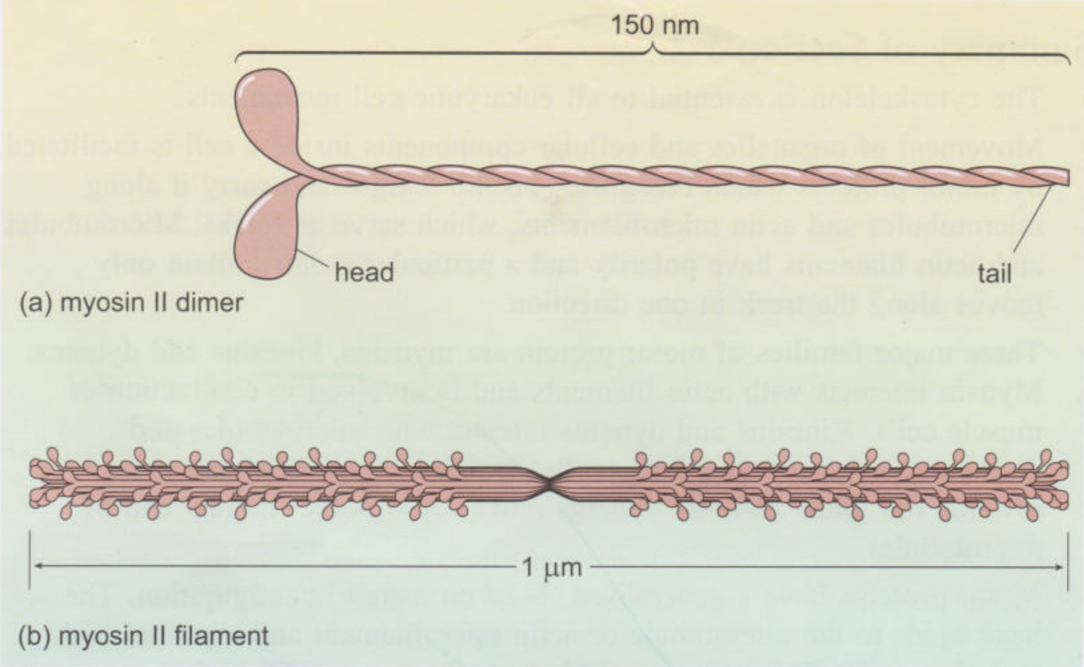
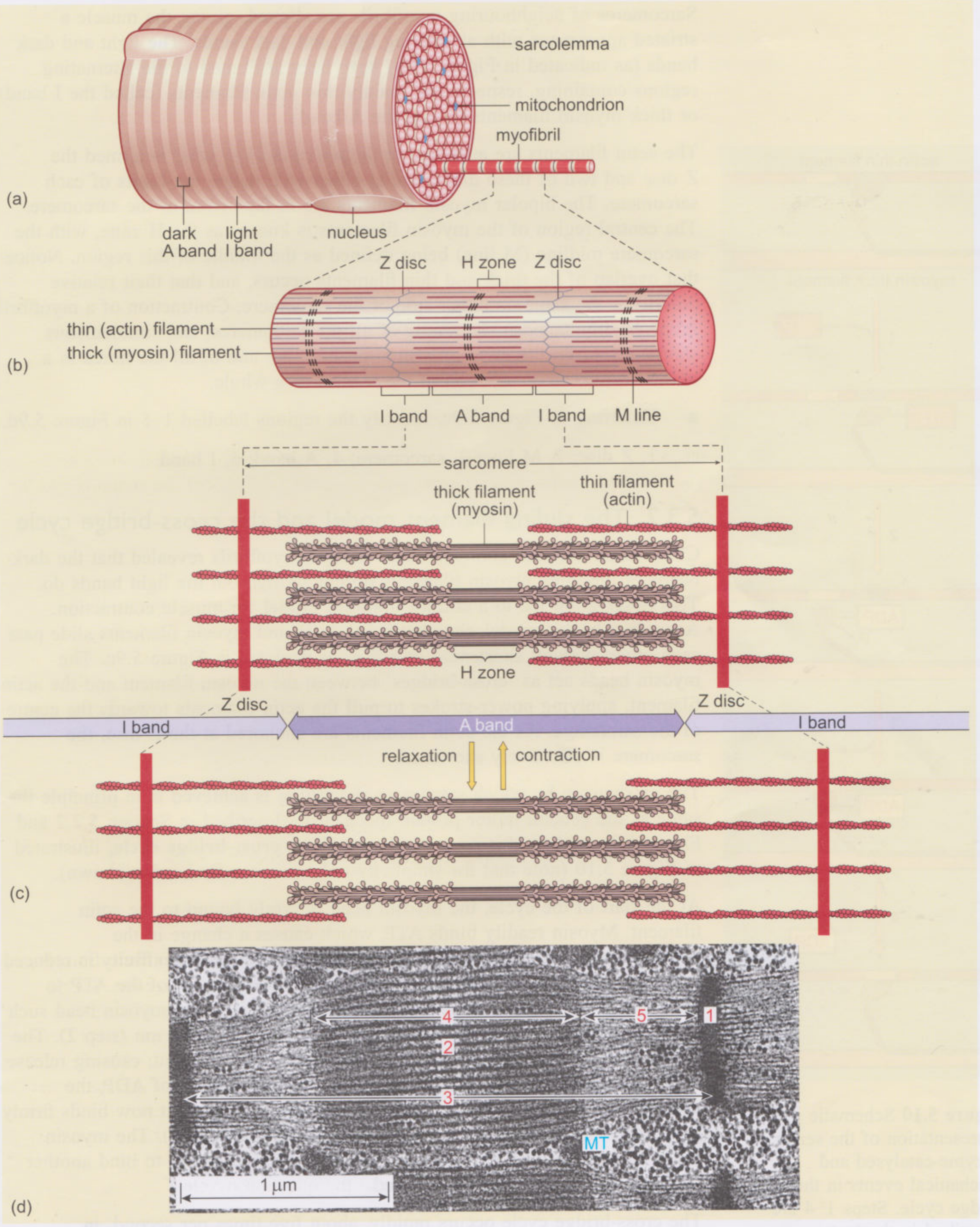


Figure 5.8 (a) A single myosin II dimer and (b) a muscle myosin filament assembled from multiple dimers. Note the globular heads of the subunits and the intertwined tails.

5.3.1 Myofibrils

The discussion here will concentrate on skeletal muscle, in which the contraction process has been particularly well characterised. Skeletal muscle is made up of long cylindrical muscle fibres (up to a few centimetres), which are multinucleate **syncytia** (singular, syncytium). These membrane-bound masses of cytoplasm and nuclei are formed during development, by the fusion of hundreds of individual cells (called myoblasts). Within a muscle fibre, the nuclei are found close to the cell membrane, referred to as the *sarcolemma*, and most of the cytoplasm is taken up with **myofibrils**, highly organised linear structures consisting of actin filaments and myosin filaments (Figure 5.9a and b). Under the microscope, myofibrils can be seen to consist of a regular series of light and dark bands in repeating units termed **sarcomeres**.

Figure 5.9 Organisation of skeletal muscle. (a) A skeletal muscle fibre is composed of many myofibrils encased in the sarcolemma together with mitochondria which supply the energy required for muscle contraction. (b) Detail of a single myofibril showing the banding arrangement. (c) Details of the thick and thin filaments in the sarcomere. Actin and myosin filaments slide past each other during contraction causing shortening of the sarcomere. The schematic shows both a contracted and a relaxed sarcomere. (d) Electron micrograph of human skeletal muscle. This image of a longitudinal section of a myofibril shows a whole sarcomere. Note the closely associated mitochondria (MT). Numbers relate to the question overleaf.


sarcolemma
mitochondrion
myofibril
(a)
dark
A band
light
I band
nucleus
Z disc
H zone
Z disc
thin (actin) filament
thick (myosin) filament
(b)
I band
A band
I band
M line
sarcomere
thick filament
(myosin)
thin filament
(actin)
H zone
Z disc
Z disc
I band
A band
I band
relaxation
contraction
(c)
4
5
1
2
3
MT
1 µm
(d)

Sarcomeres of neighbouring myofibrils are aligned, giving the muscle a striated appearance with alternating light and dark bands. The light and dark bands (as indicated in Figure 5.9b) have been attributed to the alternating regions containing, respectively, mainly thin actin filaments (called the I band) or thick myosin filaments (called the A band).

The actin filaments are anchored at their plus ends to what is termed the Z disc and two of these discs, one at either end, define the limits of each sarcomere. The bipolar myosin filaments are in the centre of the sarcomere. The central region of the myosin filaments is known as the H zone, with the sarcomere midline (M line) being defined as the middle of this region. Notice that overlap of the thick and thin filaments occurs, and that their relative polarity is the same on either side of the sarcomere. Contraction of a myofibril involves simultaneous shortening of all the sarcomeres; and simultaneous shortening of all the myofibrils in a muscle fibre and of all the fibres in a muscle results in contraction of the muscle as a whole.

■ Referring to Figure 5.9c, identify the regions labelled 1–5 in Figure 5.9d.

□ 1, Z disc; 2, M line; 3, sarcomere; 4, A band; 5, I band.

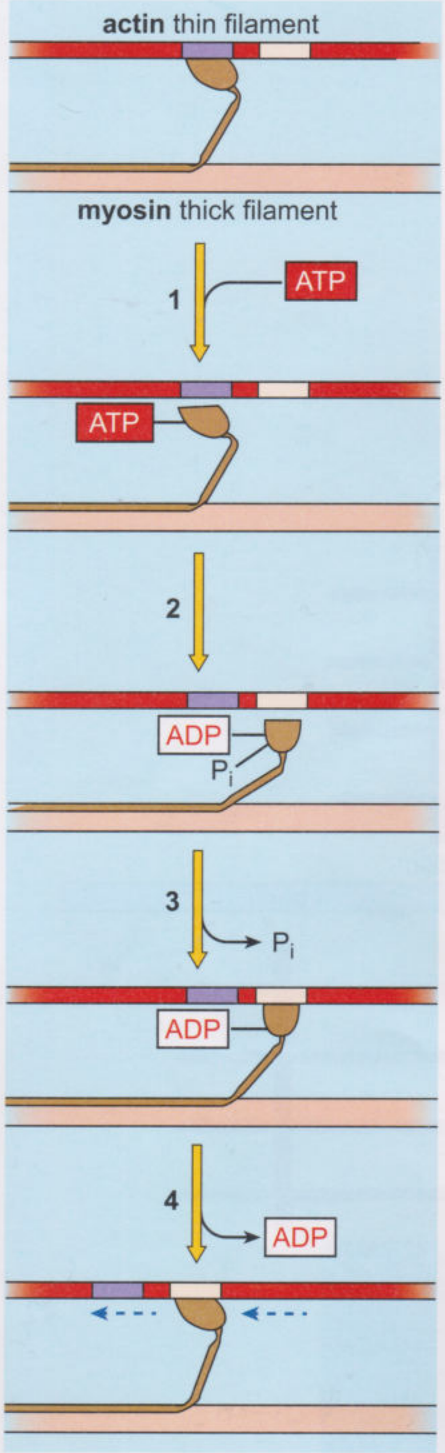


Figure 5.10 Schematic representation of the series of enzyme-catalysed and mechanical events in the cross-bridge cycle. Steps 1–4 are described in the text.

5.3.2 The sliding filament model and the cross-bridge cycle

Comparison between contracted and relaxed myofibrils revealed that the dark bands of the thick myosin filaments do not shorten but the light bands do. This observation led to a **sliding filament model** for muscle contraction. According to this model, the alternating actin and myosin filaments slide past each other, without shortening of either, as depicted in Figure 5.9c. The myosin heads act as 'cross-bridges' between the myosin filament and the actin filament, applying power-strokes to pull the actin filaments towards the centre of the sarcomere. As the actin filaments are anchored at the Z disc, the sarcomere is effectively shortened.

The mechanism by which sarcomere shortening is achieved is in principle the same as the general motor protein mechanism described in Section 5.2.2 and Figure 5.7a, and, in this context, is termed the **cross-bridge cycle**, illustrated in Figure 5.10 (note that for simplicity only one myosin head is shown).

At the start of the cycle, the myosin head is firmly bound to the actin filament. Myosin readily binds ATP, which causes a change in the conformation of the actin-binding site such that the binding affinity is reduced and the myosin head releases the actin (step 1). Hydrolysis of the ATP to ADP and P_i results in a large conformational change in the myosin head such that it is displaced along the filament by a distance of 5–10 nm (step 2). The myosin head binds weakly to a new site on the actin filament, causing release of P_i and tighter binding to the actin (step 3). With release of ADP, the myosin head reverts to its original conformation and, since it now binds firmly to the actin filament, drags the actin filament with it (step 4). The myosin head, now bound to a new part of the actin filament, is free to bind another ATP and the process can be repeated.

The cross-bridge cycle occurs rapidly, about five times per second, in thousands of myosin heads along a sarcomere, and muscle contracts fully in less than one-tenth of a second. Clearly, ATP is a prerequisite as it is necessary to weaken the interaction between the myosin head and the actin filament and to provide energy for the process. If ATP levels in the muscle are depleted, the myosin head, in the absence of bound ATP, remains tightly bound to the actin filament. The muscle therefore stalls in a rigid state called 'rigor'. This is what happens following death, when ATP levels drop and rigor mortis results.

Summary of Section 5.3

- Skeletal muscle consists of giant multinucleate syncytia called muscle fibres, which contain myofibrils – bundles of actin filaments and myosin filaments which interdigitate in a regular, repetitive fashion. The longitudinal repeat unit is termed the sarcomere.
- The sliding filament model and the cross-bridge cycle describe our current understanding of the mechanism of muscle contraction. Myosin molecules cycle, with repeated binding and hydrolysis of ATP, between different conformations with altered affinities for the actin filament. In this way, myosin repeatedly binds to the actin, dissociates and re-binds at a different site, thereby pulling the actin filament along the myosin filament and shortening the sarcomere.

5.4 Cell motility

So far this chapter has focused on intracellular movements in eukaryotic cells and how these depend on interactions between cytoskeletal components and motor proteins, interactions that are also central to contraction in muscle cells. This section is concerned with movement of whole cells from one location to another – cell motility. Cell motility is evident in both prokaryotic and eukaryotic cells. As you will see, most strikingly in eukaryotic cells, the cytoskeleton is essential to cell motility, just as it is for intracellular movements.

5.4.1 Chemotaxis

Before going on to study examples of cellular motility in more detail, it is appropriate to introduce the subject of chemotaxis, a process that is relevant to the migratory behaviour of many motile cell types. **Chemotaxis** is the term used to describe orientation and movement of a cell along a concentration gradient of a chemical, or movement in the direction of the gradient (either towards or away from the higher concentration). In a complex multicellular organism, where different cells have specialised functions, movement of a cell in relation to another is often in response to some extracellular signal produced by other cells. Often, these signals literally attract the cell (**chemoattractants**) and may be specifically produced in the target region where the moving cell is required. In some cases, a repellent signal may trigger movement of a cell away from a region.

■ How could such signals be interpreted by a responsive cell and lead to cell movement?

□ A specific receptor on the cell surface recognises and transduces the signals, initiating intracellular signalling mechanisms (Chapter 4) which regulate processes of cell movement.

Single-celled organisms, including amoebae (eukaryotic) and bacteria (prokaryotic), also demonstrate chemotactic responses to environmental stimuli and signals from other cells, although different mechanisms are involved in motility in these cells. As well as chemotactic responses to particular chemicals such as nutrients or hydrogen ions (which determine pH), some unicellular organisms respond to other stimuli such as light (phototaxis), oxygen (aerotaxis), temperature (thermotaxis) and even magnetic fields (magnetotaxis).

5.4.2 Bacterial motility and flagellar movement

The study of bacterial chemotaxis has focused primarily on *Escherichia coli* and *Salmonella typhimurium*. Many bacterial species swim, and motile species are often found in environments where nutrients are patchily distributed and motility confers a survival advantage. These bacteria possess a sensory system, which interprets signals from the environment. Generally bacteria can sense a variety of nutrients (including sugars and amino acids) and toxins or chemicals that interfere with cellular respiration, as well as pH and temperature. However, what a bacterium interprets as 'favourable' may change depending on its physiological growth stage.

The direction in which bacteria swim is essentially random, since they are too small for a gradient of a particular attractant or repellent to be detectable over the short length of the cell. They can, however, bias the direction of swimming towards favourable signals and away from unfavourable signals, by increasing **tumbling**, which changes the direction of swim. Tumbling occurs more often when repellents are sensed and is reduced when attractants are sensed. In this way, movement away from unfavourable environmental factors is more likely and conversely a movement towards favourable conditions is less likely to be interrupted (Figure 5.11).

Structure of the bacterial flagellum

The structure responsible for generating movement in most bacteria is the flagellum. The bacterial flagellum acts as a kind of propeller. Rotation requires separation of two surfaces in order to achieve sliding of one part against the other over all angles. Whereas true rotation is rare at the cellular level, at a molecular level, rotational movements can be supported by helical structures, such as cylinders and rings, which are formed by the assembly of identical polypeptide subunits. Figure 5.12 illustrates the structure and the propeller action of the flagellum in a Gram-negative bacterium.

The cell walls of Gram-negative bacteria have a double lipid bilayer (i.e. the inner, cell membrane and the outer membrane), and sandwiched between these fluid structures, in a compartment referred to as the periplasmic space, is a rigid peptidoglycan layer (Book 1, Section 3.3). Gram-positive bacteria have only one lipid bilayer (the cell membrane) and their flagellar structure differs, some of the protein rings being absent.

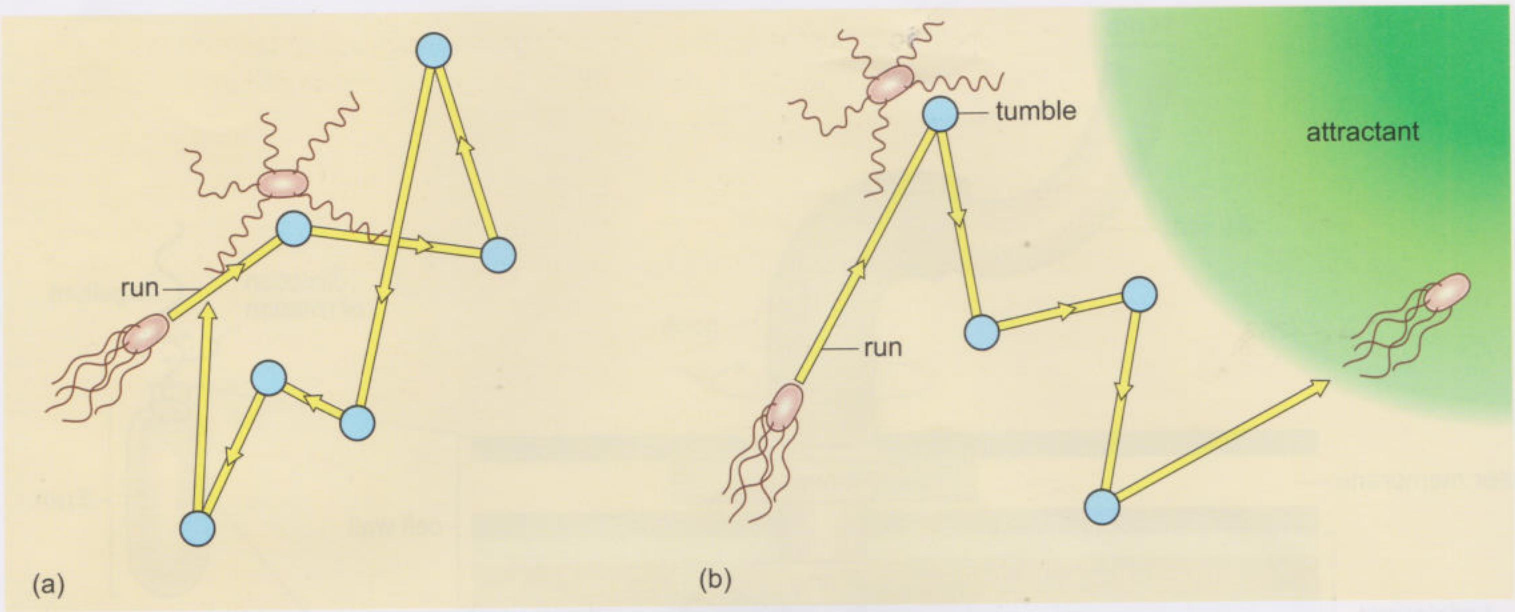


Figure 5.11 Bacterial chemotaxis. (a) In the absence of a chemical attractant (or repellent), the cell swims randomly in 'runs', changing direction during 'tumbles' (blue circles). (b) In the presence of an attractant, runs become more biased and the cell moves up the concentration gradient of the attractant.

A helical filament (about 10 μm long), which functions like a propeller blade, is attached via a small flexible hook and a rod, to protein rings called the S-M ring and C ring in the (inner) cell membrane which together act as a *rotor* (represented in Figure 5.12). Another protein ring, the P ring, is anchored in the peptidoglycan layer of the cell wall and the rotating S-M and C rings turn against it. A further protein structure, the L ring, serves as a *bearing* and also seals the outer membrane around the rotating rod. Note that although these ring structures are shown as solid shapes in Figure 5.12, they are in fact composed of a number of different proteins.

Flagellar movement

Turning of the rotor (the S-M and C rings) results in turning of the rod and hook and propagation of a wave-like motion along the flagellum. This turning process is not fully understood but it is known to be driven by a complex of a protein called Mot that surrounds the S-M and C rings and acts as a motor. The motor is powered by movement of protons (H^+) down an electrochemical gradient from the periplasmic space into the cytoplasm. The electrochemical proton gradient is established by pumping protons out of the cytoplasm, into the periplasmic space, a process that uses energy. The protons return to the cytoplasm, down their electrochemical gradient, via the Mot protein (Figure 5.12). The energy released as protons move from the periplasmic space into the cytoplasm is used to generate the movement of the rotor and hence of the flagellum.

■ What other protein complex located in a biological membrane and powered by a proton gradient have you met before? What does it do?

□ The ATP synthase, located in inner mitochondrial membrane, is powered by a proton gradient and generates ATP (Section 3.6.3).

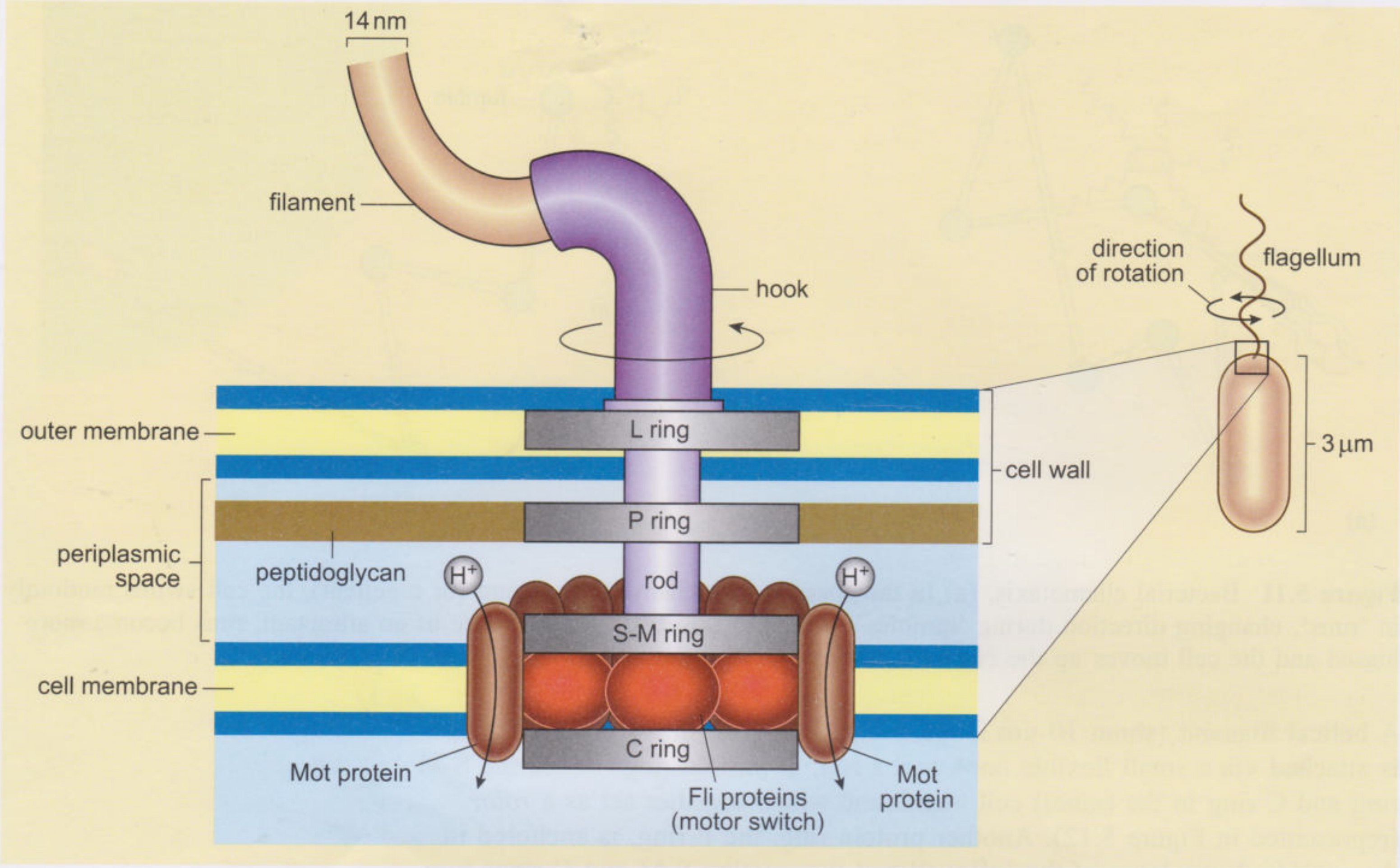


Figure 5.12 Structure of the prokaryotic flagellum and attachment to the cell wall and membranes in a Gram-negative bacterium. The L ring is embedded in the outer membrane and the P ring is embedded in the peptidoglycan layer within the periplasmic space. The S-M ring and the C ring are embedded in the cytoplasmic membrane. The Mot proteins function as the flagellar motor, whereas the Fli proteins function as a motor switch. The flagellar motor rotates the filament to propel the cell through the medium.

- ■ What is the name given to the energy stored in the electrochemical proton gradient, which drives the flagellar motor?
- □ It is a proton motive force.

A further component of the flagellum structure is a complex of proteins known as Fli, which are located in the cell membrane and closely associated with both the Mot proteins and the S-M and C rings (Figure 5.12). The Fli proteins act as a switch for the motor, determining the direction of rotation (clockwise or counterclockwise) of the rings, and hence of the flagellum. They may also be able to trigger the rod to disengage and stop rotation altogether.

A single *E. coli* or *S. typhimurium* cell usually has about 10 flagella (Figure 5.2), which can rotate at speeds of up to 300 revolutions per second, propelling the organism at a speed of several cell lengths per second. The helical nature of the flagellar filament confers a 'handedness' to the flagellum. When rotation is counterclockwise, the flagella draw together into a bundle which propels the bacterium steadily in a straight line in one direction. Reversing the direction of rotation causes the bundle of flagella to fly apart and the bacterium tumbles chaotically. A return to counterclockwise rotation of the flagella then propels the bacterium forward again in a different direction. By regulating the direction of rotation of the motor, the bacterial sensory apparatus, described below, can therefore modify the direction of movement.

Note that certain Gram-negative bacteria have internal flagella, located between the outer membrane and the cell wall. These are the spirochaetes, which include *Leptospira* species, which cause leptospirosis, and *Borrelia burgdorferi*, which causes Lyme disease in humans. A number of internal flagella form what are known as axial filaments that extend from both ends of the bacterium. Rotation of the internal flagella causes a corkscrew type of movement which propels the bacterium through the surrounding fluid.

Chemotactic regulation of flagellar movement

How does a change in some environmental factor modulate the rotation of the bacterial flagellum? Receptors located in the cell membrane of the bacteria detect changes in specific environmental factors. In Gram-negative bacteria, these receptors generally span the cell membrane which separates the periplasmic space from the cytoplasm. Water- and lipid-soluble attractant or repellent molecules have unrestricted access to the periplasmic space, where they bind to the periplasmic domain of specific receptors.

Signalling from the sensory receptors is via a cascade of regulatory proteins, illustrated in Figure 5.13. The receptors that bind the chemoattractant or repellent are known as **methyl-accepting chemotaxis proteins (MCPs)** because their activity is regulated by methylation. The MCPs have a periplasmic domain that binds the ligand (attractant or repellent), a transmembrane domain and a highly conserved cytosolic domain that interacts with a series of downstream signalling proteins called **Che proteins** (pronounced 'key', for **che**motaxis). MCPs that recognise attractants and repellents have a common downstream signalling pathway; however, crucially, *MCPs that bind a repellent are activated while those that bind an attractant are inactivated.*

An intermediate protein called CheW, which has no known catalytic function but serves as a 'scaffolding' or 'linking' protein in the assembly of the signalling protein complex, is required for activation of a protein kinase called CheA. When an MCP is activated, it causes CheA to autophosphorylate. Phosphorylated CheA (CheA-P) transfers its phosphate group to another protein in the cascade, called CheY, and phosphorylated CheY (CheY-P) interacts with a Fli protein at the base of the flagellum and causes the flagellar motor to change direction or stop, with the result that the bacterium stops swimming and tumbles (see above).

■ What would happen to levels of CheA-P if the bacterium experiences: (i) an increase in the concentration of a repellent, or (ii) a decrease in the concentration of an attractant?

□ In both cases CheA-P levels would increase because MCPs that can bind the repellent would become activated while MCPs that can bind the attractant would be unoccupied and hence activated.

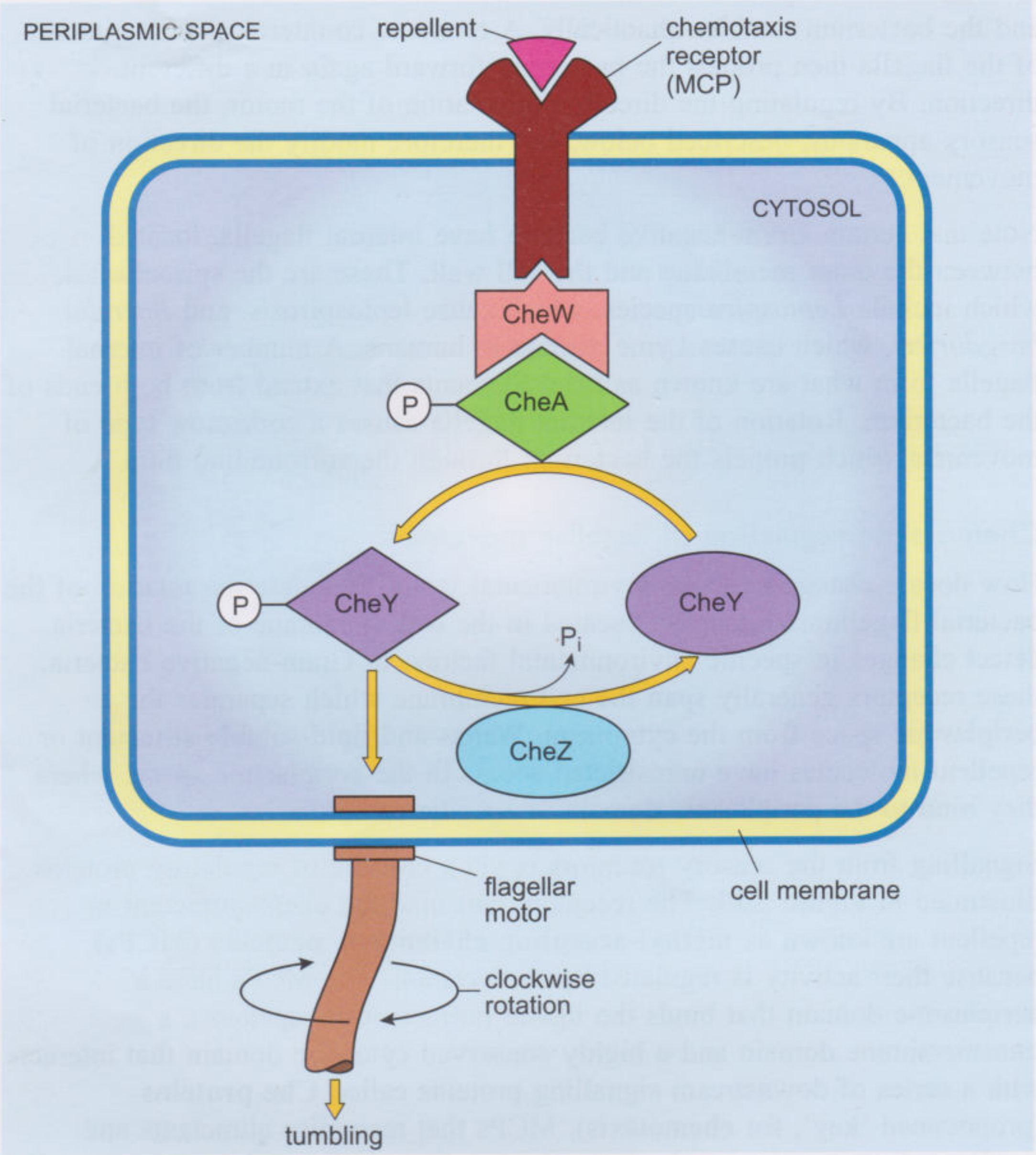


Figure 5.13 Chemotactic regulation of bacterial flagellar movement. Note that the figure depicts regulation of flagellar movement in Gram-negative bacteria, though similar mechanisms operate in Gram-positive bacteria. A ligand (attractant/ repellent), in the periplasmic space, binds to a specific transmembrane receptor (MCP). MCPs that bind a repellent (as shown here) are activated while those that bind an attractant are inactivated. Through a cascade of signalling events, flagellar rotation can be stopped or its direction reversed. Methylation of MCPs modulates this response, as described in the text.

Thus a reduction in the level of an extracellular chemoattractant (or an increase in an extracellular repellent) results in increased tumbling and an increased frequency of changes of swimming direction of the bacterium. When extracellular levels of a chemoattractant increase (or levels of a repellent decrease), MCP activation is reduced, CheA becomes dephosphorylated, and phosphorylated CheY levels decrease. Only the phosphorylated form of CheY can interact with the Fli proteins to affect rotation; thus, while the bacterium is moving towards an attractant (or away from a repellent), intracellular levels of CheY-P remain low, rotation continues in the same direction and the direction of swim is unchanged. CheY-P is recycled to its unphosphorylated form (i.e. dephosphorylated) by a phosphatase called CheZ, ensuring that the system remains sensitive to change (Figure 5.13).

Methylation of MCPs is an important regulatory aspect of the sensory system. The degree of methylation depends on the activities of two further proteins called CheR and CheB. CheR is a methyltransferase enzyme that methylates specific glutamate residues of the MCPs (i.e. it adds a $-CH_3$ group), whereas CheB is a methylesterase enzyme that removes these modifications. Regulation of MCPs is, therefore, an example of reversible covalent modification, as described in Section 1.8.5.

As a bacterium moves towards increasing concentrations of attractant, more methyl groups are added to the MCP proteins. Methylation has the effect of desensitising the bacterium to the attractant, so that tumbling, repressed by swimming towards the attractant, is restored. This adaptation means that the bacterium can sense a much wider range of concentration differences and remains responsive to changes in the level of the attractant.

5.4.3 Creeping movements of eukaryotic cells

Many motile cells, including some unicellular protists and many different animal cells grown in culture, move by creeping or crawling over surfaces. The movements involved in creeping actions are also common to the processes of phagocytosis and endocytosis and are dependent on actin filaments. Three processes are involved, represented in Figure 5.14:

1. Pushing out protrusions at the front of the cell (termed its leading edge).
2. Adherence of the protrusions to the surface over which the cell is moving (which may be, for example, extracellular matrix or neighbouring cells).
3. Application of traction on the points of adherence at the front of the cell pulls the rear end free (retraction), so that the cell moves forward and forms new protrusions at the leading edge.

Most studies of creeping cell movement have been performed on cells in culture, moving across a surface or substrate, whether a plastic culture vessel, a coating of extracellular matrix proteins or a layer of cells. In this sort of study, the extension of protrusions from the leading edge is clearly seen in the form of filopodia (Figure 5.15) and lamellipodia. **Filopodia** (singular **filopodium**) are thin finger-like extensions, about 0.1 μm wide and 5–10 μm long. **Lamellipodia** (singular **lamellipodium**) are thin sheet-like extensions. These extensions are rapidly extended and retracted and seem to explore the environment.

Both filopodia and lamellipodia contain actin filaments, oriented such that their plus end points towards the cell surface. Addition of actin monomers to the end of the growing filament is facilitated by a complex of proteins at the plus end. Extension of the actin filament pushes the membrane out to form the protrusions (Figure 5.14).

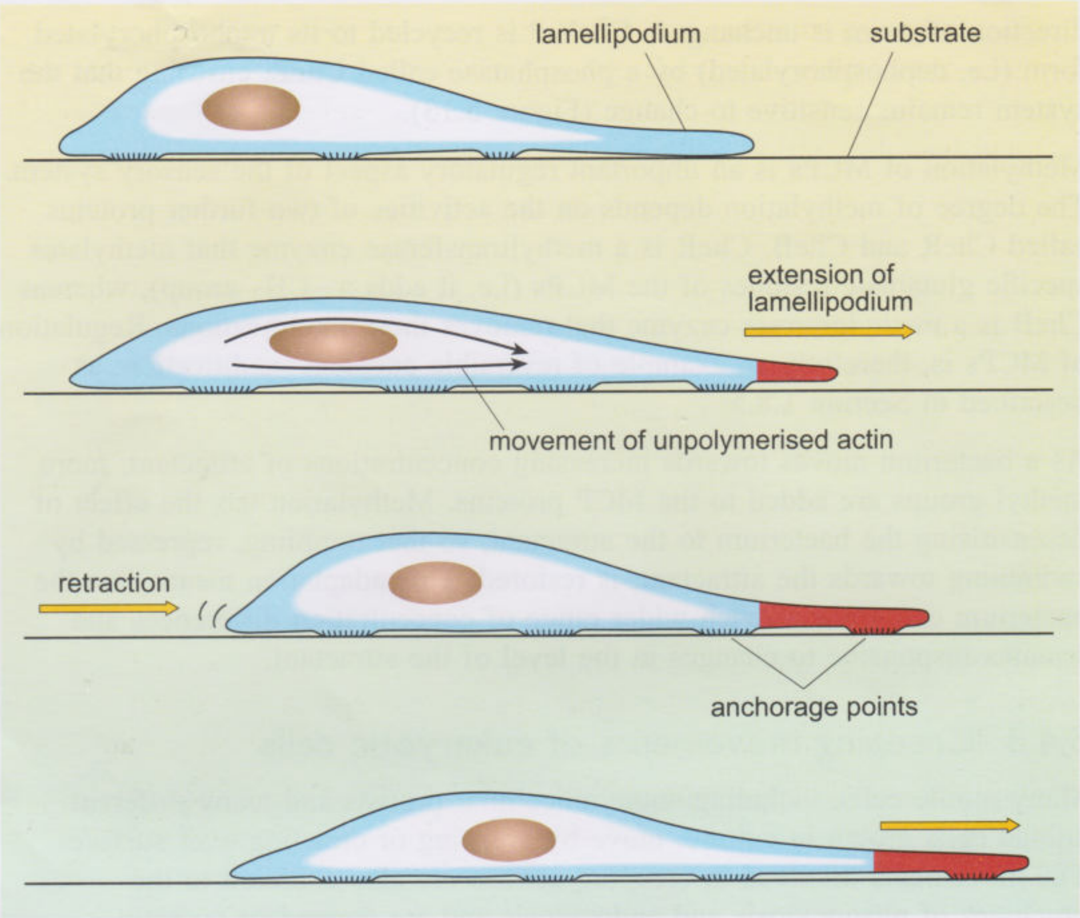


Figure 5.14 Schematic of cell creeping by extension and adherence of a protrusion to a surface, and retraction of the trailing part of the cell.

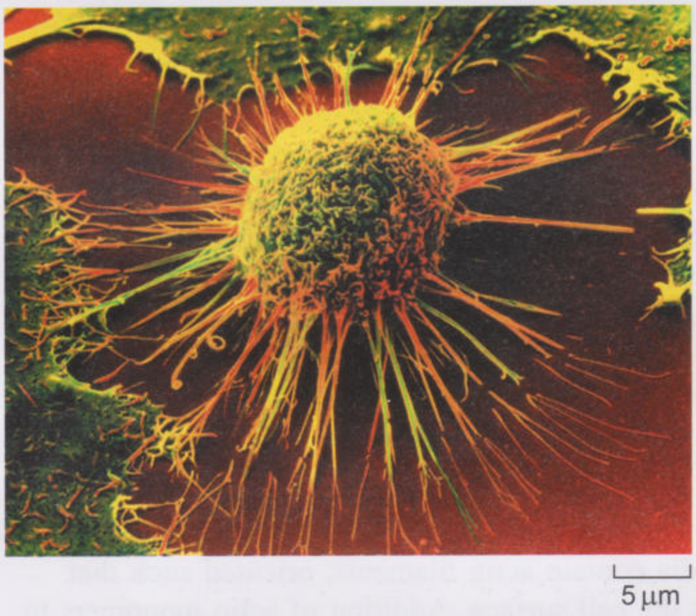


Figure 5.15 Scanning EM of a cancer cell in culture showing filopodia.

- The drug cytochalasin caps the plus ends of actin filaments. What effect would this drug have on creeping cell movement in eukaryotic cells?

□ Creeping cell movement in eukaryotic cells requires dynamic assembly and disassembly of actin for the extension and retraction of filopodia and lamellipodia. Capping of the actin filaments would prevent their extension and would inhibit movement.

Adherence of the leading edge to the surface is mediated by cell adhesion molecules, including integrins (Book 1, Section 3.5.1, and Section 2.3.5 in this book) that bind specifically to proteins in the extracellular matrix or to proteins on the surface of neighbouring cells. The cytosolic domain of integrins interacts with the cell cytoskeleton, capturing actin filaments of the cortex (the network of microfilaments that lies inside the cell membrane), thereby anchoring the cell to the substrate. Contraction of the actin filaments then pulls the trailing part of the cell towards the leading edge and the point of adherence. The contraction of actin filaments is dependent on motor proteins of the myosin family.

Creeping cell movements require integration of intracellular processes controlling the attachment of cell adhesion molecules (to the extracellular matrix or to other cells) and those that control the organisation of the cytoskeleton. One important group of proteins that affect the organisation of the cytoskeleton are the **Rho-family GTPases**, which is a subgroup of the small GTPase family of which Ras is a member (Section 4.7.1). Activation of Rho family GTPases leads to reorganisation of the cytoskeleton as well as affecting cell adhesion, for example by influencing interactions between integrins and the actin network.

Chemotactic regulation in eukaryotic cells

Chemotactic responses in eukaryotic cells are mediated by G protein-coupled receptors (GPCRs; Section 4.6) on the cell surface. In multicellular animals there is a wide range of types of extracellular signalling molecule and of mechanisms of GPCR activation. The largest group of chemotactic molecules in these organisms are the chemokines, with at least 70 members being identified in mammals.

Most chemokines are small proteins with M_r of approximately 10 000. They were originally identified for their roles in directing the movement of immune cells in the immune response; however, they are also involved in many developmental processes.

5.4.4 Eukaryotic flagella and cilia

Flagella are not limited to prokaryotes, being found on some eukaryotic cells such as sperm cells of some multicellular animals and on some protists; however, in eukaryotes they have a more complex structure than do prokaryotic flagella and have an entirely different motor mechanism. Cilia extend from the surface of many different animal cell types and from a group of protists termed ciliates (Figure 5.1c). Cilia have a similar structure to that of eukaryotic flagella, though they are shorter (about 10–15 μm long, whereas flagella can be over 50 μm in length). In animals, their function is to propel fluid over the surface of the cell: for example, cilia in the epithelium lining the respiratory tract force mucus and debris up towards the mouth. They also can propel cells through a fluid environment, as in the movement of the ovum from the ovary to the womb via the Fallopian tubes in female mammals. Under the scanning electron microscope, the fields of cilia lining the respiratory tract look like a dense carpet (Figure 5.1b). These large numbers of cilia bend in the same direction, in a coordinated fashion. The movement generated in a cilium is whip-like, cycling in 0.1–0.2 seconds; whereas in a eukaryotic flagellum, slower wave-like movements drive the cell forward during swimming.

Structure of the eukaryotic flagellum and cilium

The **axoneme** is the general term for the macromolecular protein complex that characterises both cilia and flagella of eukaryotic cells and is also often referred to as the '9+2' structure because of the arrangement of microtubules that give it its overall shape (Figure 5.16). There are nine outer microtubule doublets (i.e. two microtubules associated along their lengths) arranged around two central singlet microtubules, and the microtubules are continuous for the entire length of the structure. The 9+2 organisation is apparent when the axoneme is viewed in cross-section, as in the electron micrograph in Figure 5.16a.

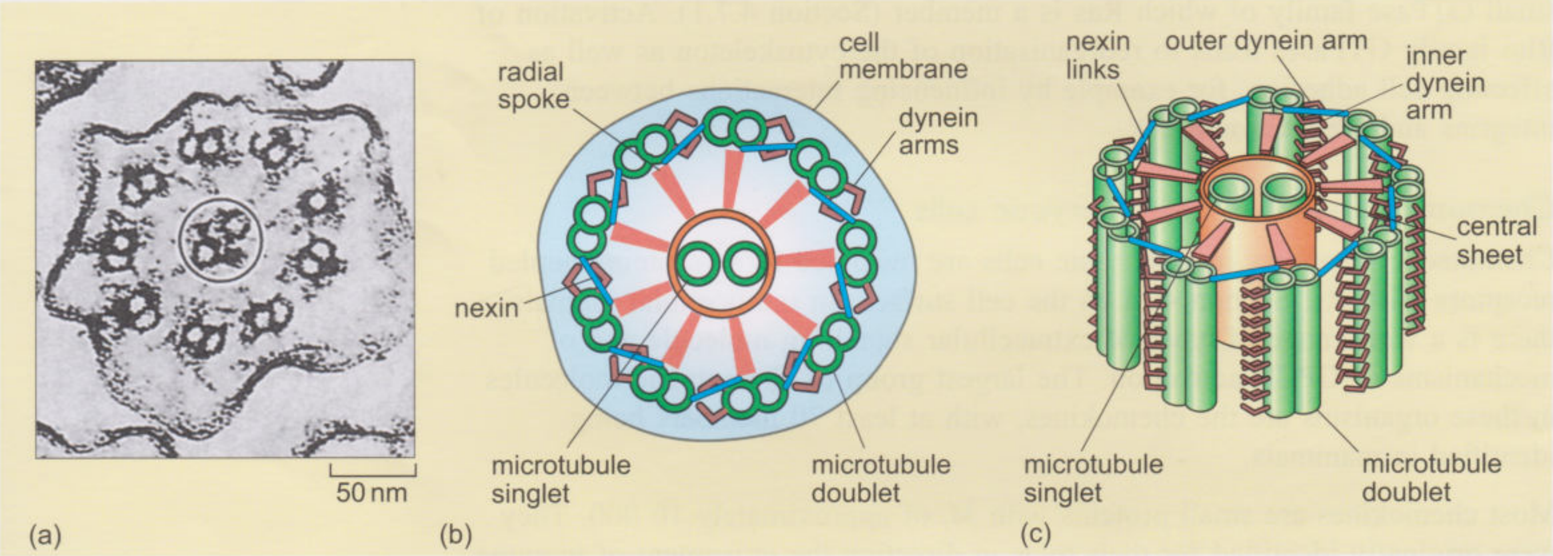


Figure 5.16 (a) Electron micrograph of the axoneme of the protist *Tetrahymena thermophila* seen in cross-section. (b) Schematic cross-section of an axoneme. (c) Three-dimensional reconstruction of the arrangement of proteins that make up the axoneme.

As the schematic representations in Figure 5.16b and c show, the microtubule doublets that form the outer ring are linked by a protein called nexin, which is spaced at intervals along the microtubules; and dynein motor proteins are associated along the length of the doublets, extending from one side towards the neighbouring doublet. The two central microtubules are surrounded by a sheath, and radial spokes composed of several types of protein project from this sheath towards the doublets. The structure as a whole is enclosed by a continuation of the cell membrane; thus eukaryotic flagella and cilia are regarded as cellular organelles.

Cilia and flagella end inside the cell in a structure called the **basal body**, depicted in Figure 5.17. The microtubule doublets of the axoneme extend into the basal body where they are associated with a third microtubule, forming triplets; however, the central two singlet microtubules do not extend into the basal body. Thus a cross-section of the basal body reveals a ring consisting of nine triplets of microtubules with no central core structure.

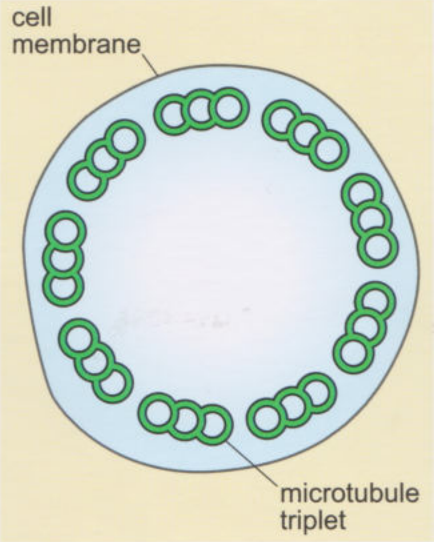


Figure 5.17 Schematic cross-section through the basal body.

Mechanism of axonemal movement

Unlike the bacterial flagellum, where movement is driven by a motor at its base, the axonemal movement is propagated by the structure itself. 10–15% of the protein mass is attributable to dynein motor protein, which hydrolyses ATP to generate axonemal movement. The dynein molecules are arranged, in pairs, along the length of each microtubule doublet and extend like arms towards neighbouring microtubule doublets with which they can reversibly bind.

It is sliding of the outer microtubule doublets relative to each other, through the activity of dynein, which makes the axoneme as a whole move. Because the doublets are linked by nexin, the sliding movement causes the structure to bend, as depicted in Figure 5.18.

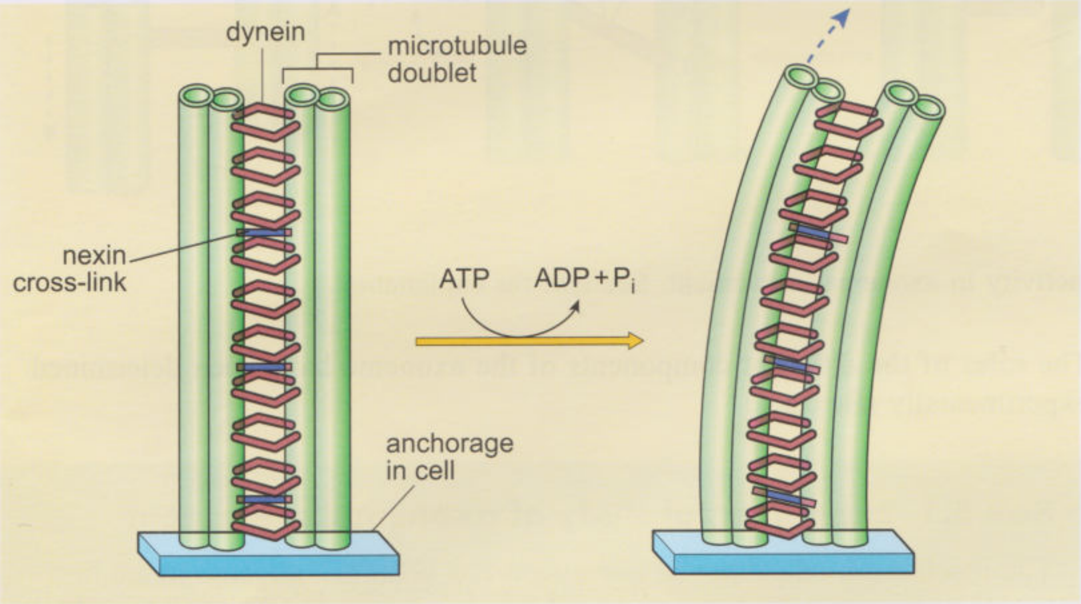


Figure 5.18 Bending of microtubules in the axoneme occurs because nexin cross-links (blue) restrict the movement of sliding microtubules.

The dynein motor proteins, projecting from each microtubule doublet towards its neighbour, generate the force necessary to slide one doublet alongside another. This process requires ATP, which is hydrolysed by dynein to ADP and P_i. Each dynein molecule can hydrolyse about 50 ATP molecules per second. As described in the general cycle of motor protein activity (Section 5.2.2), dynein undergoes conformational changes upon binding and hydrolysis of ATP and on release of ADP. The conformational changes in dynein are associated with changes in the motor protein's affinity for the neighbouring microtubule doublet. This process is illustrated in Figure 5.19 and can be summarised as follows:

1. In the absence of ATP, the dynein associated with doublet X extends towards, and binds to, the adjacent doublet, Y.
2. On binding ATP, the dynein releases microtubule Y.
3. Hydrolysis of the ATP to ADP and P_i results in another conformational change, such that the dynein binds to microtubule Y again but at a different position, pointing downward, towards the basal body.
4. With release of ADP and P_i, dynein reverts to its original conformation, thereby moving the adjacent doublet.

In this way, dynein motors all along the length of the microtubule doublets generate the sliding force and, since microtubule movement is restricted by the nexin cross-links, the axoneme bends. Successive bending and relaxation of the axoneme generates the wave-like motion observed in the eukaryotic flagellum or cilium.

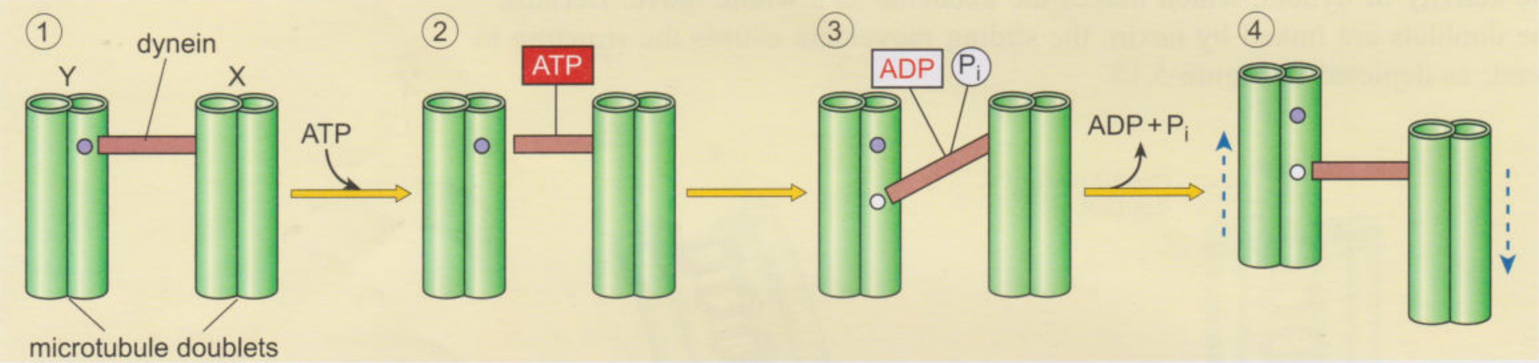


Figure 5.19 Dynein motor protein activity in axonemal movement. See text for explanation.

The roles of the different components of the axoneme have been determined experimentally (Box 5.1).

Box 5.1 Experimental study of axonemal movement

The mechanism of axonemal movement has been investigated in studies involving experimental dissection of the components of the flagellum, illustrated in Figure 5.20.

The membrane around the flagellum can be removed by incubation with a mild detergent. Without the membrane, the internal environment is lost and ATP is no longer available. As a result, movement ceases. However, movement can be restored by the addition of ATP, demonstrating that the axoneme is still functional in the absence of its enclosing membrane.

Mild digestion with a proteolytic enzyme such as trypsin destroys the nexin cross-links between the microtubule doublets and the radial spokes. In the presence of ATP, the microtubules can still slide over each other but, without the restriction of the nexin links, this sliding action does not result in bending of the axoneme. The sliding process eventually releases individual microtubule doublets and irreversibly dissociates the axoneme structure.

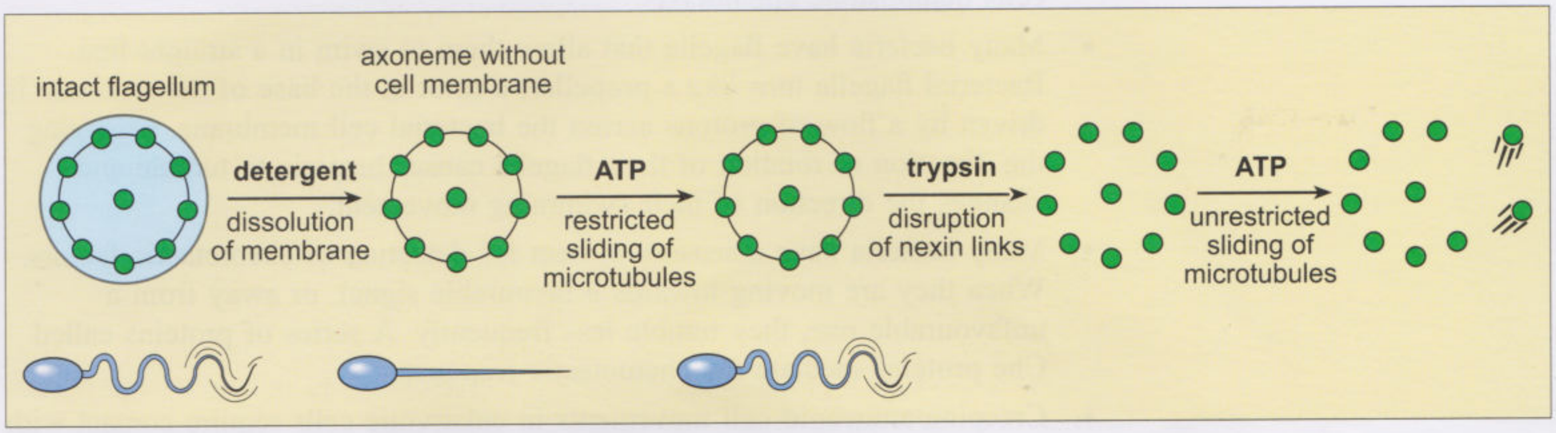

intact flagellum
axoneme without cell membrane
detergent
dissolution of membrane
ATP
restricted sliding of microtubules
trypsin
disruption of nexin links
ATP
unrestricted sliding of microtubules

Figure 5.20 Experimental dissection of the axoneme.

The control of cilia movement in *Paramecium*

Ciliates, for example *Paramecium* (Figure 5.1c), are covered by cilia that beat backwards in unison to propel the organism in one direction. As the *Paramecium* moves through water it rotates on its long axis, and small particles of food are swept into the gullet (a cavity in which food particles are gathered for consumption by phagocytosis). If a *Paramecium* encounters an obstacle, it reverses the beating of the cilia to swim backwards, turns, and moves off forward again in a different direction.

The direction in which the cilia beat is controlled by calcium channels in the cell membrane. When the cell bumps into an obstacle there is a change in the cell membrane potential, calcium channels in the cell membrane open and the cell depolarises as calcium ions (Ca^{2+}) rush into the cell.

■ In what example of cell signalling have you already encountered ion channels that open in response to changes in membrane potential?

□ In nerve axons, where nerve impulses, or action potentials, are generated by depolarisation of the cell via Na^{+} channels (Section 2.8.4).

Calcium ions act as an intracellular second messenger (Section 4.6.3) to transduce a signal that switches the cilia into beating in a forward direction, allowing the cell to swim backwards. Not all the cilia switch smoothly into the same direction, which usually results in a random change of direction. When the cell re-establishes the membrane potential, the cilia return to beating backwards and the cell resumes forward swimming. Cilia in other organisms are also coordinated by similar mechanisms involving ion channels.

Summary of Section 5.4

- Chemotaxis is orientation of a cell along a concentration gradient of a chemical or movement in the direction of the gradient (either towards or away from the higher concentration). Many prokaryotic and eukaryotic cells demonstrate chemotaxis.
- Many bacteria have flagella that allow them to swim in a straight line. Bacterial flagella turn like a propeller; a rotor at the base of the structure is driven by a flow of protons across the bacterial cell membrane. Changing the direction of rotation of their flagella causes bacteria to tumble and changes the direction of their swimming movement.
- Many bacteria have a sensory system for detection of chemotactic factors. When they are moving towards a favourable signal, or away from a unfavourable one, they tumble less frequently. A series of proteins called Che proteins mediate the chemotactic response.
- Creeping/amoeboid cell movements in eukaryotic cells require contact with a solid substrate and depend on actin filaments and myosin. Actin-containing filopodia and lamellipodia push forward at the leading edge of the cell and adhesion to the substrate is mediated by integrins. Contraction of actin filaments, brought about by myosin, pulls the trailing part of the cell towards the leading edge.
- Flagella and cilia also produce movement in many unicellular eukaryotes, as well as in specialised cell types in higher organisms. They have a common '9+2' assembly of microtubules, termed the axoneme.
- Eukaryotic flagellar movement is wave-like and is produced by sliding of the microtubules of the axoneme in relation to each other. This sliding movement requires ATP and is generated by the motor protein dynein. Restriction of microtubule sliding by nexin cross-links causes the structure to bend, and repeated cycles generate the wave-like motion.

5.5 Final word

Cell motility is essential for survival of many unicellular organisms and, in multicellular organisms, cell movement is an important aspect of function in specialised cell types such as phagocytic cells, bronchial epithelium and sperm cells, as well as being a feature of general cell function.

As you will learn in Book 3, Chapter 1, internal movement of cellular contents and rearrangements and/or changes in cell shape are a significant feature of the eukaryotic cell cycle and differentiation. The rest of Book 3 examines cellular responses to environmental challenges and disease and explores some examples of the biotechnological exploitation of cells.

5.6 Learning outcomes

5.1 Describe the molecular and structural characteristics of cytoskeletal components involved in movement in eukaryotic cells.

5.2 Describe the generalised mechanism by which motor proteins generate movement, and illustrate these processes with specific examples from the following: the sliding filament model of skeletal muscle contraction; eukaryotic flagellar movement; intracellular movement of eukaryotic organelles.

5.3 Compare and contrast flagellar structure and movement in eukaryotic and prokaryotic cells.

5.4 Describe the intracellular signalling cascade that controls bacterial chemotaxis.

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S294 Cell biology
Science: Level 2

● Book 1 Generating Diversity
○ **Book 2** **Working Cells**
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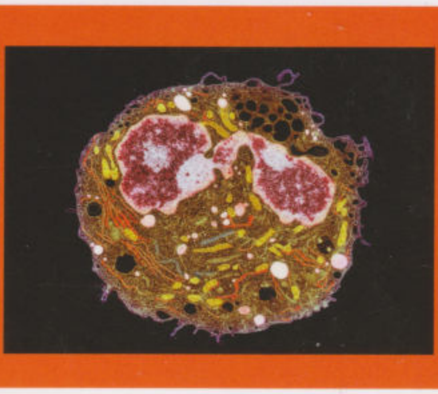

A false colour transmission electron micrograph of a human white blood cell, in this case an eosinophil. Numerous subcellular structures can be seen in this image; the largest structure in the cell is the nucleus, which has the lobed appearance characteristic of eosinophils.

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